Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
S16	601	(alpha adj amylase$1) same bacillus same (mutant$1 or variant$1)	US-PGPUB; USPAT	OR	OFF	2004/03/22 12:39
S17	1884	(mutant$1 or variant$1) near10 (stability or thermostabilty or calcium adj depend$8)	US-PGPUB; USPAT	OR	OFF	2004/03/22 12:40
S18	97	S16 and S17	US-PGPUB; USPAT	OR	OFF	2004/03/22 12:40

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DOCUMENT-IDENTIFIER: US 20040048351 A1

TITLE: Alpha-amylase mutants

PUBLICATION-DATE: March 11, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
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APPL-NO: 10/ 644187

DATE FILED: August 20, 2003

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child 10186042 20020628 US

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parent-patent 6436888 US

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parent-patent 6143708 US

child 09182859 19981029 US

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COUNTRY	APPL-NO	DOC-ID	APPL-DATE
DK	0515/96	1996DK-0515/96	April 30, 1996
DK	0712/96	1996DK-0712/96	June 28, 1996
DK	0775/96	1996DK-0775/96	July 11, 1996
DK	1263/96	1996DK-1263/96	November 8, 1996

US-CL-CURRENT: 435/204, 510/226 , 510/320

ABSTRACT:

The invention relates to a variant of a parent Termamyl-like a-amylase, which variant has a-amylase activity and exhibits an alteration in at least one of the following properties relative to said parent a-amylase: substrate

specificity, substrate binding, substrate cleavage pattern, thermal stability, pH/activity profile, pH/stability profile, stability towards oxidation, Ca.sup.2+ dependency and specific activity.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a division of Ser. No. 10/186,042, filed on Jun. 27, 2002, which is a division of Ser. No. 09/672,459, filed on Sep. 28, 2000 (now a U.S. Pat. No. 6,436,888), which is a continuation of Ser. No. 09/182,859, filed on Oct. 29, 1998 (now U.S. Pat. No. 6,143,708), which is a continuation of PCT/DK97/00197 filed Apr. 30, 1997 which claims priority under 35 U.S.C. 119 of Danish applications 0515/96 filed Apr. 30, 1996, 0712/96 filed Jun. 28, 1996, 0775/96 filed Jul. 11, 1996, and 1263/96 filed Nov. 8, 1996, the contents of which are fully incorporated herein by reference.

---------- KWIC ---------

Pre-Grant Publication Document Identifier - DID (1):
US 20040048351 A1

Summary of Invention Paragraph - BSTX (5):
[0004] Among more recent disclosures relating to .alpha.-amylases, WO 96/23874 provides three-dimensional, X-ray crystal structural data for a Termamyl-like .alpha.-amylase which consists of the 300 N-terminal amino acid residues of the B. amyloliquefaciens .alpha.-amylase comprising the amino acid sequence shown in SEQ ID No. 4 herein and amino acids 301-483 of the C-terminal end of the B. licheniformis .alpha.-amylase comprising the amino acid sequence shown in SEQ ID No. 2 herein (the latter being available commercially under the tradename Termamyl.TM.), and which is thus closely related to the industrially important Bacillus .alpha.-amylases (which in the present context are embraced within the meaning of the term "Termamyl-like .alpha.-amylases", and which include, inter alia, the B. licheniformis, B. amyloliquefaciens and B. stearothermophilus .alpha.-amylases). WO 96/23874 further describes methodology for designing, on the basis of an analysis of the structure of a parent Termamyl-like .alpha.-amylase, variants of the parent Termamyl-like .alpha.-amylase which exhibit altered properties relative to the parent.

Summary of Invention Paragraph - BSTX (178):
[0175] Furthermore, it is preferred that the mutagenesis is carried out by use of doped or spiked oligonucleotides. The doping is preferably done so as to introduce amino acids contributing to improved stability at low pH and reduced calcium dependency at low pH of the resulting .alpha.-amylase variant. Furthermore, when selecting the doping scheme, the possibility of introducing Asn and Gln residues should generally be avoided, since Asn and Gln residues in general are associated with instability at low pH. Preferably, when a Pro residue can be inserted with potential benefits (e.g. as assessed from protein-structural considerations), the doping scheme is prepared to include a preference for introduction of a Pro residue.

Summary of Invention Paragraph - BSTX (180):
[0177] In relation to the above, a further aspect of the present invention relates to a method for generating a variant of a parent Termamyl-like .alpha.-amylase, which variant exhibits increased stability at low pH and at low calcium concentration relative to the parent, the method comprising:

Summary of Invention Paragraph - BSTX (208):
[0205] .alpha.-Amylase activity is detected by Cibacron Red labelled

amylopectin, which is immobilized on agarose. For screening for variants with increased thermal and high-pH stability, the filter with bound .alpha.-amylase variants is incubated in a buffer at pH 10.5 and 600 or 65.degree. C. for a specified time, rinsed briefly in deionized water and placed on the amylopectin-agarose matrix for activity detection. Residual activity is seen as lysis of Cibacron Red by amylopectin degradation. The conditions are chosen to be such that activity due to the .alpha.-amylase having the amino acid sequence shown in SEQ ID No. 2 can barely be detected. Stabilized variants show, under the same conditions, increased colour intensity due to increased liberation of Cibacron Red.

Summary of Invention Paragraph - BSTX (215):
[0212] In the vector, the DNA sequence should be operably connected to a suitable promoter sequence. The promoter may be any DNA sequence which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. Examples of suitable promoters for directing the transcription of the DNA sequence encoding an .alpha.-amylase variant of the invention, especially in a bacterial host, are the promoter of the lac operon of E. coli, the Streptomyces coelicolor agarase gene dagA promoters, the promoters of the Bacillus licheniformis .alpha.-amylase gene (amyL), the promoters of the Bacillus stearothermophilus maltogenic amylase gene (amyM), the promoters of the Bacillus amyloliquefaciens .alpha.-amylase (amyQ), the promoters of the Bacillus subtilis xylA and xylB genes etc. For transcription in a fungal host, examples of useful promoters are those derived from the gene encoding A. oryzae TAKA amylase, Rhizomucor miehei aspartic proteinase, A. niger neutral .alpha.-amylase, A. niger acid stable .alpha.-amylase, A. niger glucoamylase, Rhizomucor miehei lipase, A. oryzae alkaline protease, A. oryzae triose phosphate isomerase or A. nidulans acetamidase.

Detail Description Paragraph - DETX (16):
[0326] 3. Decide on which kind of mutations should be carried out, e.g. with respect to the desired stability and/or performance of the variant to be constructed

Detail Description Paragraph - DETX (87):
[0395] Construction, by Localized Random, Doped Mutagenesis, of Termamyl-like .alpha.-amylas Variants Having an Improved Stability at Low pH and a Reduced Dependency on Calcium Ions for Stability Compared to the Parent Enzyme

Detail Description Paragraph - DETX (90):
[0398] has a very satisfactory stability at low pH and low calcium concentrations. In an attempt to further improve the stability at low pH and low calcium concentration of said .alpha.-amylase variant random mutagenesis in preselected regions wase performed.

Detail Description Paragraph - DETX (114):
[0421] The mutations indicated in bold were introduced by the random mutagenesis method. The stability data for these variants appear from Table 11 in Example 3.

Detail Description Paragraph - DETX (140):
[0445] This example summarises the stability results of variants characterised by a fluorimetric assay at 70.degree. C. under two different conditions, (1) pH 4.5 and 1 mM CaCl.sub.2 and (2) pH 6.2 and 10 .mu.M CaCl.sub.2.

Claims Text - CLTX (2):

2. A variant according to claim 1, exhibiting increased stability at low pH and low Ca.sup.2+ concentration relative to the parent Termamyl-like .alpha.-amylase, and comprising mutations selected from the following: H156Y+A181T+A209V; H156Y+A181T+N190F+A209V+Q264S; A1*+N2*+L3V+M15T+R23K+S-29A+A30E+Y31H+A33S+E34D+H35I+H156Y+A181T+A2 09V; A1*+N2*+L3V+M1ST+R23K+S29-A+A30E+Y31H+A33S+E34D+H35I+H156Y+A181T+N1 90F+A209V; A1*+N2*+L3V+M15T+R23K+S29A+A30E+Y31H+A33S+E34D+H35I+H156Y+A181T+N1 90F+A209V+Q264S.

Claims Text - CLTX (3):

3. A variant according to claim 1 or 2, wherein the parent Termamyl-like .alpha.-amylase is selected from: the B. licheniformis .alpha.-amylase having the sequence shown in SEQ ID No. 2, the B. amyloliquefaciens .alpha.-amylase having the sequence shown in SEQ ID No. 4, the B. stearothermophilus .alpha.-amylase having the sequence shown in SEQ ID No. 6, the Bacillus strain NCIB 12512 .alpha.-amylase having the sequence shown in FIGS. 1 and 2, the Bacillus strain NCIB 12513 .alpha.-amylase having the sequence shown in FIG. 2, and the Bacillus sp. #707 .alpha.-amylase having the sequence shown in FIG. 2.

Claims Text - CLTX (23):

23. A method for generating a variant of a parent Termamyl-like .alpha.-amylase, which variant exhibits increased stability at low pH and at low calcium concentration relative to the parent, the method comprising: (a) subjecting a DNA sequence encoding the parent Termamyl-like .alpha.-amylase to random mutagenesis, (b) expressing the mutated DNA sequence obtained in step (a) in a host cell, and (c) screening for host cells expressing a mutated .alpha.-amylase which has increased stability at low pH and low calcium concentration relative to the parent .alpha.-amylase.

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PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040048247 A1

TITLE: Pullulanase variants and methods for preparing such variants with predetermined properties

PUBLICATION-DATE: March 11, 2004

INVENTOR-INFORMATION:

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DK	PA20000045	2000DK-PA20000045	January 12, 2000

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371-DATE:
102(E)-DATE:

US-CL-CURRENT: 435/6, 435/210 , 435/320.1 , 435/325 , 435/69.1 , 536/23.2 , 703/11

ABSTRACT:

The present invention relates to a method for producing a variant of a parent pullulanase, the variant having at least one altered property as compared to the parent pullulanase. The invention also relates to pullulanase variants and to the use of pullulanase variants of the invention for use in particular starch conversion processes.

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US 20040048247 A1

Summary of Invention Paragraph - BSTX (2):
[0001] The present invention relates to variants of pullulanases and to methods for constructing such variants with altered properties, including stability (e.g., thermostability), pH dependent activity, substrate specificity, such as increased isoamylase activity, or specific activity; specific activity at a given pH and/or altered substrate specificity, such as

an altered pattern of substrate cleavage or an altered pattern of substrate inhibition.

Detail Description Paragraph - DETX (87):
[0115] In one embodiment, the pullulanase variant of the invention has an improved thermostability (and/or the method of the invention provides a pullulanase with an improved thermostability) as defined by differential scanning calorimetry (DSC) using the method described herein.

Detail Description Paragraph - DETX (88):
[0116] In another embodiment, the pullulanase variant of the invention has an improved thermostability (and/or the method of the invention provides a pullulanase with an improved thermostability) as defined by an increased half-time (T.sub.1/2) of at least about 5%, preferably at least about 10%, more preferably at least about 15%, more preferably at least about 25%, most preferably at least about 50%, such as at least about 100%, in the "T.sub.1/2 assay for liquefaction" described herein, using a pH of 5.0 and a temperature of 95.degree. C. Pullulanase variants according to this definition are suitable for use in the liquefaction step of the starch conversion process.

Detail Description Paragraph - DETX (90):
[0118] In a further embodiment, the enzyme variant of the invention has an improved thermostability (and/or the method of the invention provides a pullulanase with an improved thermostability) as defined by an increased half-time (T.sub.1/2) of at least about 5%, preferably at least about 10%, more preferably at least about 15%, more preferably at least about 25%, most preferably at least about 50%, such as at least about 100%, in the "T.sub.1/2 assay for saccharification" described herein, using a pH of 4.5 and a temperature of 70.degree. C. Such variants are suitable for use in the saccharification step of the starch conversion process.

Detail Description Paragraph - DETX (126):
[0154] Pullulanas Variants with Altered Stability

Detail Description Paragraph - DETX (127):
[0155] A variant with improved stability (typically increased thermostability) may be obtained by substitution with proline, substitution of histidine with another amino acid, introduction of a disulfide bond, removal of a deamidation site, altering a hydrogen bond contact, filling in an internal structural cavity with one or more amino acids with bulkier side groups, introduction of interdomain interactions, altering charge distribution, helix capping, or introduction of a salt bridge.

Detail Description Paragraph - DETX (158):
[0186] Furthermore, it is envisaged from the structure that deletion of certain amino acid residues will confer increased stability, such as increased thermostability, to the thus produced variant. Variants, which are believed to be of particular importance, comprises a deletion of amino acid residues corresponding to the following residues of the amino acid sequence set forth in SEQ ID NO: 1:

Detail Description Paragraph - DETX (160):
[0188] Other deletions which are expected to confer increased stability, such as increased thermostability, to the pullulanase variant comprises a deletion of amino acid residues corresponding to the following residues of the amino acid sequence set forth in SEQ ID NO: 1:

Detail Description Paragraph - DETX (162):
[0190] Furthermore, the following deletions are expected to confer increased

stability, such as increased thermostability, to the pullulanase variant comprises a deletion of amino acid residues corresponding to the following residues of the amino acid sequence set forth in SEQ ID NO: 1:

Detail Description Paragraph - DETX (165):
[0193] For example, it is envisaged that deletion of the below amino acid residues will confer increased stability, such as increased thermostability, to the thus produced variant of the pullulanase from Bacillus deramificans (SEQ ID NO: 3):

Detail Description Paragraph - DETX (167):
[0195] Other deletions which are expected to confer increased stability, such as increased thermostability, to the pullulanase variant comprises a deletion of amino acid residues corresponding to the following residues of the amino acid sequence set forth in SEQ ID NO: 3:

Detail Description Paragraph - DETX (169):
[0197] Furthermore, the following deletions are expected to confer increased stability, such as increased thermostability, to the pullulanase variant comprises a deletion of amino acid residues corresponding to the following residues of the amino acid sequence set forth in SEQ ID NO: 3:

Detail Description Paragraph - DETX (182):
[0210] f) testing the stability and/or the temperature dependent activity profile of the variant; and

Detail Description Paragraph - DETX (184):
[0212] h) selecting a variant having increased stability and/or an altered temperature dependent activity profile as compared to the parent pullulanase.

Detail Description Paragraph - DETX (185):
[0213] In a preferred embodiment of the invention the variant pullanase provided by the above method have increased thermostability as compared to the parent pullulanase. The thermostability of a given variant may be assessed as described in the above section entitled "Methods for determining stability, activity and specificity".

Detail Description Paragraph - DETX (193):
[0221] A variant with improved stability (typically improved thermostability) as compared to the parent pullulanase may be obtained by introducing new interdomain and intradomain contacts, such as establishing inter- or intradomain disulfide bridges.

Detail Description Paragraph - DETX (200):
[0228] f) testing the stability of said variant; and

Detail Description Paragraph - DETX (202):
[0230] h) selecting a variant having increased stability as compared to the parent pullulanase.

Detail Description Paragraph - DETX (203):
[0231] In a preferred embodiment of the invention the variant pullanase provided by the above method have increased thermostability as compared to the parent pullulanase. The thermostability of a given variant may be assessed as described in the above section entitled "Methods for determining stability, activity and specificity".

Detail Description Paragraph - DETX (208):
[0236] A variant with improved stability (typically improved

thermostability) as compared to the parent pullulanase may be obtained by changing the surface charge distribution of the pullulanase. For example, when the pH is lowered to about 5 or below histidine residues typically become positively charged and, consequently, unfavorable electrostatic interactions on the protein surface may occur. By engineering the surface charge of the pullulanase one may avoid such unfavorable electrostatic interactions that in turn leads to a higher stability of the pullulanase.

Detail Description Paragraph - DETX (215):
[0243] f) testing the stability of said variant; and

Detail Description Paragraph - DETX (217):
[0245] h) selecting a variant having increased stability as compared to the parent pullulanase.

Detail Description Paragraph - DETX (225):
[0253] In a preferred embodiment of the invention the variant pullulanase provided by the above method(s) have increased thermostability as compared to the parent pullulanase. The thermostability of a given variant may be assessed as described in the above section entitled "Methods for determining stability, activity and specificity".

Detail Description Paragraph - DETX (233):
[0261] Variants with improved stability, in particular variants with improved thermostability, can be obtained by improving existing or introducing new interdomain or intradomain contacts. Such improved stability can be achieved by the modifications listed below.

Detail Description Paragraph - DETX (234):
[0262] Thus, one preferred embodiment of the invention relates to a variant of a parent pullulanase which has an improved stability and one or more salt bridges as compared to the parent pullulanase, wherein said variant comprises a modifications, e.g., a substitution, in a position corresponding to at least one of the following sets of positions in SEQ ID NO: 1: 301, 385, 298, 299, 385 and 299+385, in particular L301R, N385R, H298R, N299R, N385D and N299R+N385D.

Detail Description Paragraph - DETX (403):
[0431] In relation to the above, a further aspect of the present invention relates to a method for generating a variant of a parent pullulanase, wherein the variant exhibits an altered property, such as increased thermostability, increased stability at low pH and at low calcium concentration, relative to the parent pullulanase, the method comprising:

Detail Description Paragraph - DETX (425):
[0453] 3. Decide on which kind of mutations should be carried out, e.g. with respect to the desired stability and/or performance of the variant to be constructed

Detail Description Paragraph - DETX (438):
[0466] In the vector, the DNA sequence should be operably connected to a suitable promoter sequence. The promoter may be any DNA sequence that shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. Examples of suitable promoters for directing the transcription of the DNA sequence encoding a pullulanase variant of the invention, especially in a bacterial host, are the promoter of the lac operon of E. coli, the Streptomyces coelicolor agarase gene dagA promoters, the promoters of the Bacillus licheniformis .alpha.-amylase gene (amyL), the promoters of the Bacillus stearothermophilus maltogenic amylase gene (amyM), the promoters of the

Bacillus amyloliquefaciens alpha-amylase (amyQ), the promoters of the Bacillus subtilis xylA and xylB genes, etc. For transcription in a fungal host, examples of useful promoters are those derived from the gene encoding A. oryzae TAKA amylase, Rhizomucor miehei aspartic proteinase, A. niger neutral alpha-amylase, A. niger acid stable alpha-amylase, A. niger glucoamylase, Rhizomucor miehei lipase, A. oryzae alkaline protease, A. oryzae triose phosphate isomerase (TPI) or A. nidulans acetamidase.

Detail Description Paragraph - DETX (454):

[0482] To screen for variants with increased stability, the filter with bound pullulanase variants can be pretreated prior to the detection step described above to inactivate variants that do not have improved stability relative to the parent pullulanase. This inactivation step may consist of, but is not limited to, incubation at elevated temperatures in the presence of a buffered solution at any pH from pH 2 to 12, and/or in a buffer containing another compound known or thought to contribute to altered stability, e.g., surfactants, EDTA, EGTA, wheat flour components, or any other relevant additives. Filters so treated for a specified time are then rinsed briefly in deionized water and placed on plates for activity detection as described above. The conditions are chosen such that stabilized variants show increased enzymatic activity relative to the parent after incubation on the detection media.

Detail Description Paragraph - DETX (455):

[0483] To screen for variants with altered thermostability, filters with bound variants are incubated in buffer at a given pH (e.g., in the range from pH 2-12) at an elevated temperature (e.g., in the range from 50.degree.-110.degree. C.) for a time period (e.g., from 1-20 minutes) to inactivate nearly all of the parent pullulanase, rinsed in water, then placed directly on a detection plate containing immobilized Cibacron Blue labeled pullulan and incubated until activity is detectable. As will be understood, thermostability and increased isoamylase activity may be tested simultaneously by using a detection plate containing immobilized Cibacron Red labeled amylopectin and incubate until activity is detectable. Moreover, pH dependent stability can be screened for by adjusting the pH of the buffer in the above inactivation step such that the parent pullulanase is inactivated, thereby allowing detection of only those variants with increased stability at the pH in question. To screen for variants with increased calcium-dependent stability, calcium chelators, such as ethylene glycol-bis(beta-aminoethyl ether) N,N,N',N'-tetraacetic acid (EGTA), is added to the inactivation buffer at a concentration such that the parent pullulanase is inactivated under conditions further defined, such as buffer pH, temperature or a specified length of incubation.

Detail Description Paragraph - DETX (456):

[0484] The variants of the invention may be suitably tested by assaying the pullulan- or amylopectin-degrading activity of the variant, for instance by growing host cells transformed with a DNA sequence encoding a variant on a starch-containing agarose plate and identifying pullulan- and/or amylopectin-degrading host cells as described above. Further testing in regard to altered properties, including specific activity, substrate specificity, cleavage pattern, thermoactivation, thermostability, pH dependent activity or optimum, pH dependent stability, temperature dependent activity or optimum, transglycosylation activity, stability, and any other parameter of interest, may be performed on purified variants in accordance with methods known in the art as described below.

Detail Description Paragraph - DETX (457):

[0485] Finally the present invention relates to the used of a pullulanase

variant of the invention for starch conversion, both for the liquefaction and saccharification steps, in particular for producing syrups, such as dextrose or maltose syrups. A pullulanase variant of the invention may also be used for producing sweeteners; ethanol, such as fuel, drinking and industrial ethanol, from starch or whole grains (see for instance U.S. Pat. No. 5,231,017-A or U.S. Pat. No. 5,756,714-A hereby incorporated by reference). Further, a pullulanase variant of the invention may also be used as cleaning ingredient, in laundry detergent compositions, dishwashing detergent, and hard surface cleaning compositions (see e.g., WO 99/23211, WO 97/07202 or WO 96/238874 for details on examples on cleaning compositions ingredients, the references hereby being incorporated by reference). Normally a cleaning or detergent composition also comprises at least a protease, in particular Bacillus proteases, and also one or more of the following activities: alpha-amylase, lipase, cellulase, mannanase, CGTase, maltogenic amylase.

Detail Description Paragraph - DETX (480):

[0504] The fermentation supernatant containing pullulanase variant is subjected to the stability assay (Thermostability Assay 2 (T1/2)) in order to determine T1/2 values of inactivation using the assay described above.

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TITLE: Alpha-amylase mutants

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INVENTOR-INFORMATION:

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Kjaerulff, Soren	Vanlose		DK	

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child 09769864 20010125 US

parent division-of 09183412 19981030 US GRANTED

parent-patent 6204232 US

non-provisional-of-provisional 60064662 19971106 US

non-provisional-of-provisional 60093234 19980717 US

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COUNTRY	APPL-NO	DOC-ID	APPL-DATE
DK	1240/97	1997DK-1240/97	October 30, 1997
DK	1998 00936	1998DK-1998 00936	July 14, 1998

US-CL-CURRENT: 435/202

ABSTRACT:

The invention relates to a variant of a parent Termamyl-like .alpha.-amylase, which exhibits an alteration in at least one of the following properties relative to said parent .alpha.-amylase: i) improved pH stability at a pH from 8 to 10.5; and/or ii) improved Ca.sup.2+ stability at pH 8 to 10.5, and/or iii) increased specific activity at temperatures from 10 to 60.degree. C.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a divisional of U.S. application Ser. No. 09/769,864, filed on Jan. 25, 2001, which is a divisional of U.S. application Ser. No. 09/183,412, filed on Oct. 30, 1998, and claims priority under 35 U.S.C. 119 of Danish application no. 1240/97, filed on Oct. 30, 1997, Danish application no. PA 1998 00936, filed on Jul. 14, 1998, U.S. provisional application No. 60/064,662, filed on Nov. 6, 1997 and U.S. provisional application No. 60/093,234, filed on Jul. 17, 1998, the contents of which are fully incorporated herein by reference.

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Pre-Grant Publication Document Identifier - DID (1):
US 20040038368 A1

Summary of Invention Paragraph - BSTX (6):
[0005] Among more recent disclosures relating to .alpha.-amylases, WO 96/23874 provides three-dimensional, X-ray crystal structural data for a Termamyl-like .alpha.-amylase which consists of the 300 N-terminal amino acid residues of the B. amyloliquefaciens .alpha.-amylase (BAN.TM.) and amino acids 301-483 of the C-terminal end of the B. licheniformis .alpha.-amylase comprising the amino acid sequence (the latter being available commercially under the tradename Termamyl.TM.), and which is thus closely related to the industrially important Bacillus .alpha.-amylases (which in the present context are embraced within the meaning of the term "Termamyl-like .alpha.-amylases", and which include, inter alia, the B. licheniformis, B. amyloliquefaciens (BAN.TM.) and B. stearothermophilus (BSG.TM.) .alpha.-amylases). WO 96/23874 further describes methodology for designing, on the basis of an analysis of the structure of a parent Termamyl-like .alpha.-amylase, variants of the parent Termamyl-like .alpha.-amylase which exhibit altered properties relative to the parent.

Summary of Invention Paragraph - BSTX (14):
[0012] Alterations in properties which may be achieved in variants(mutants) of the invention are alterations in: the stability of the Termamyl-like .alpha.-amylase at a pH from 8 to 10.5, and/or the Ca.sup.2+ stability at pH 8 to 10.5, and/or the specific activity at temperatures from 10 to 60.degree. C., preferably 20-50.degree. C., especially 30-40.degree. C.

Detail Description Paragraph - DETX (49):
[0170] Preferred high pH stability variants include one or more of the following substitutions in the SP722 .alpha.-amylase (having the amino acid sequence shown in SEQ ID NO: 2):

Detail Description Paragraph - DETX (53):
[0174] .alpha.-amylase variants with improved stability at high pH can be constructed by making substitutions in the regions found using the molecular dynamics simulation mentioned in Example 2. The simulation depicts the region(s) that has a higher flexibility or mobility at high pH (i.e., pH 8-10.5) when compared to medium pH.

Detail Description Paragraph - DETX (77):
[0198] In a preferred embodiment the variant is the Bacillus strain NCIB 12512 .alpha.-amylase with deletions in D183 and G184 and further one of the following substitutions: R181Q,N and/or G182T,S,N and/or D183*; G184* and/or K185A,R,D,C,E,Q,G,H,I,L,M,N,F,P,S,T,W,Y,V and/or A186T,S,N,I,V and/or W189T,S,N,Q and/or N195F and/or N270R,D and/or E346Q and/or K385R and/or K458R and/or P459T.

Detail Description Paragraph - DETX (172):
[0293] In the vector, the DNA sequence should be operably connected to a suitable promoter sequence. The promoter may be any DNA sequence which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. Examples of suitable promoters for directing the transcription of the DNA sequence encoding an .alpha.-amylase variant of the invention, especially in a bacterial host, are the promoter of the lac operon of E. coli, the Streptomyces coelicolor agarase gene dagA promoters, the promoters of the Bacillus licheniformis .alpha.-amylase gene (amyL), the promoters of the Bacillus stearothermophilus maltogenic amylase gene (amyM), the promoters of the Bacillus amyloliquefaciens .alpha.-amylase (amyQ), the promoters of the Bacillus subtilis xylA and xylB genes etc. For transcription in a fungal host, examples of useful promoters are those derived from the gene encoding A. oryzae TAKA amylase, Rhizomucor miehei aspartic proteinase, A. niger neutral .alpha.-amylase, A. niger acid stable .alpha.-amylase, A. niger glucoamylase, Rhizomucor miehei lipase, A. oryzae alkaline protease, A. oryzae triose phosphate isomerase or A. nidulans acetamidase.

Detail Description Paragraph - DETX (211):
[0331] The assay can be used to screening of Termamyl-like .alpha.-amylase variants having an improved stability at high pH compared to the parent enzyme and Termamyl-like .alpha.-amylase variants having an improved stability at high pH and medium temperatures compared to the parent enzyme depending of the screening temperature setting

Detail Description Paragraph - DETX (242):
[0362] 3. Decide on which kind of mutations should be carried out, e.g. with respect to the desired stability and/or performance of the variant to be constructed

Detail Description Paragraph - DETX (266):
[0383] Method of Extracting Important Regions for Identifying .alpha.-Amylase Variants with Improved pH Stability and Altered Temperature Activity

Detail Description Paragraph - DETX (269):
[0386] 1. The approach used for extracting important regions for identifying .alpha.-amylase variants with high pH stability:

Detail Description Paragraph - DETX (270):
[0387] The important regions for constructing variants with improved pH stability are the regions which at the extreme pH display the highest mobility, i.e., regions having the highest isotropic fluctuations.

Detail Description Paragraph - DETX (277):
[0393] Construction, by Localized Random, Doped Mutagenesis, of Termamyl-Like .alpha.-Amylase Variants Having an Improved Ca2+ Stability at Medium Temperatures Compared to the Parent Enzyme

Detail Description Paragraph - DETX (408):
[0520] Determination of pH Stability at Alkaline pH of Variants of the Parent .alpha.-Amylase Having the Amino Acid Sequence Shown in SEQ ID NO:2.

Detail Description Paragraph - DETX (416):
[0527] Determination of Calcium Stability at Alkaline pH of Variants of the Parent .alpha.-Amylase Having the Amino Acid Sequence Shown in SEQ ID NO: 1, SEQ ID NO: 2 and SEQ ID NO: 4.

Detail Description Paragraph - DETX (417):
[0528] A: Calcium Stability of Variants of the Sequence in SEQ ID NO: 1

Detail Description Paragraph - DETX (421):
[0532] B: Calcium Stability of Variants of the Sequence in SEQ ID NO: 2

Detail Description Paragraph - DETX (428):
[0539] C: Calcium Stability of Variants of the Sequence in SEQ ID NO: 4

Claims Text - CLTX (6):
6. The variant according to any of claims 1-5, exhibiting improved stability at pH 8 to 10.5, having mutations in one or more of the position(s) corresponding to the following positions (using SEQ ID NO: 2 numbering): T141, K142, F143, D144, F145, P146, G147, R148, G149, R181, A186, S193, N195, K269, N270, K311, K458, P459, T461.

Claims Text - CLTX (9):
9. The variant according to claims 1-5, exhibiting improved Ca.sup.2+ stability at pH 8 to 10.5, having mutations in one or more of the following positions (using the SEQ ID NO: 2 numbering): R181, G182, D183, G184, K185, A186, W189, N195, N270, E346, K385, K458, P459.

Claims Text - CLTX (12):
12. A variant according to claims 1-11, wherein the parent Termamyl-like .alpha.-amylase is selected from: the Bacillus strain NCIB 12512 .alpha.-amylase having the sequence shown in SEQ ID NO: 1; the B. amyloliquefaciens .alpha.-amylase having the sequence shown in SEQ ID NO: 5; the B. licheniformis .alpha.-amylase having the sequence shown in SEQ ID NO: 4.

PGPUB-DOCUMENT-NUMBER: 20040023349

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040023349 A1

TITLE: Processes for making ethanol

PUBLICATION-DATE: February 5, 2004

INVENTOR-INFORMATION:
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APPL-NO: 10/ 460455

DATE FILED: June 12, 2003

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60388488 20020613 US

US-CL-CURRENT: 435/161

ABSTRACT:

The present invention provides improved processes for recovering components of distillers' grain, such as, the components of distillers' dried grain (DDG), for use in various applications, including in the production of ethanol.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. provisional application No. 60/388,488 filed Jun. 13, 2002, the contents of which are fully incorporated herein by reference

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Pre-Grant Publication Document Identifier - DID (1):
US 20040023349 A1

Detail Description Paragraph - DETX (17):
[0030] Preferred are alpha-amylases of fungal or bacterial origin. More preferably, the alpha-amylase is a Bacillus alpha-amylases, such as, derived from a strain of B. licheniformis, B. amyloliquefaciens, and B. stearothermophilus. Other alpha-amylases include alpha-amylase derived from a strain of the Bacillus sp. NCIB 12289, NCIB 12512, NCIB 12513 or DSM 9375, all of which are described in detail in WO 95/26397, and the alpha-amylase described by Tsukamoto et al., Biochemical and Biophysical Research Communications, 151 (1988), pp. 25-31. Other alpha-amylase variants and hybrids are described in WO 96/23874, WO 97/41213, and WO 99/19467. Other alpha-amylase includes alpha-amylases derived from a strain of Aspergillus, such as, Aspergillus oryzae and Aspergillus niger alpha-amylases. In a preferred embodiment, the alpha-amylase is a acid alpha-amylase. In a more preferred embodiment the acid alpha-amylase is an acid fungal alpha-amylase or

an acid bacterial alpha-amylase. More preferably, the acid alpha-amylase is an acid fungal alpha-amylase derived from the genus Aspergillus. In a preferred embodiment, the alpha-amylase is an acid alpha-amylase. The term "acid alpha-amylase" means an alpha-amylase (E.C. 3.2.1.1) which added in an effective amount has activity at a pH in the range of 3.0 to 7.0, preferably from 3.5 to 6.0, or more preferably from 4.0-5.0.

Detail Description Paragraph - DETX (25):

[0038] Other Aspergillus glucoamylase variants include variants to enhance the thermal stability: G137A and G139A (Chen et al. (1996), Prot. Engng. 9, 499-505); D257E and D293E/Q (Chen et al. (1995), Prot. Engng. 8, 575-582); N182 (Chen et al. (1994), Biochem. J. 301, 275-281); disulphide bonds, A246C (Fierobe et al. (1996), Biochemistry, 35, 8698-8704; and introduction of Pro residues in position A435 and S436 (Li et al. (1997), Protein Engng. 10, 1199-1024. Other glucoamylases include Talaromyces glucoamylases, in particular, derived from Talaromyces emersonii (WO 99/28448), Talaromyces leycettanus (U.S. Pat. No. Re. 32,153), Talaromyces duponti, Talaromyces thermophilus (U.S. Pat. No. 4,587,215). Bacterial glucoamylases contemplated include glucoamylases from the genus Clostridium, in particular C. thermoamylolyticum (EP 135,138), and C. thermohydrosulfuricum (WO 86/01831).

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PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040002142 A1

TITLE: Glucoamylase variants

PUBLICATION-DATE: January 1, 2004

INVENTOR-INFORMATION:

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APPL-NO: 10/ 421586

DATE FILED: April 23, 2003

RELATED-US-APPL-DATA:

child 10421586 A1 20030423

parent continuation-of 09612489 20000707 US ABANDONED

non-provisional-of-provisional 60143313 19990712 US

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
DK	1999 00999	1999DK-1999 00999	July 9, 1999

US-CL-CURRENT: 435/101, 435/105 , 435/115 , 435/126 , 435/144 , 435/161 , 435/205 , 435/254.3 , 435/320.1 , 536/23.2

ABSTRACT:

The invention relates to a variant of a parent fungal glucoamylase, which exhibits altered properties, in particular improved thermal stability and/or increased specific activity.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation of U.S. application Ser. No. 09/612,489 filed Jul. 7, 2000, and claims the benefit under 35 U.S.C. 119 of of U.S. provisional application No. 60/143,313 filed Jul. 12, 1999, and priority of Danish application no. PA 1999 00999, filed Jul. 9, 1999, the contents of which are fully incorporated herein by reference.

---------- KWIC ---------

Abstract Paragraph - ABTX (1):
The invention relates to a variant of a parent fungal glucoamylase, which exhibits altered properties, in particular improved thermal stability and/or

increased specific activity.

Pre-Grant Publication Document Identifier - DID (1):
US 20040002142 A1

Summary of Invention Paragraph - BSTX (2):
[0002] The present invention relates to novel glucoamylase variants (mutants) of parent AMG with altered properties, in particular with improved thermal stability and/or increased specific activity, which variants are, e.g., suitable for starch conversion, in particular for producing glucose from starch, and for ethanol production, sweetener production. More specifically, the present invention relates to glucoamylase variants and the use of such variant enzymes.

Summary of Invention Paragraph - BSTX (14):
[0012] The inventors of the present invention have provided a number of variants of a parent glucoamylase with improved thermal stability and/or increased specific activity. The improved thermal stability is obtained by mutating, e.g., by substituting and/or deleting, inserting selected positions in a parent glucoamylase. This will be described in details below.

Detail Description Paragraph - DETX (25):
[0057] In still another aspect, the invention relates to a variant of a parent glucoamylase with improved thermal stability, in particular in the range from 40-80.degree. C., preferably 63-75.degree. C., in particular at pH 4-5, using maltodextrin as the substrate, said variant comprising one or more mutations in the following positions in the amino acid sequence shown in SEQ ID NO: 2: 59, 66, 72, 119, 189, 223, 227, 313, 340, 342, 352, 379, 386, 393, 395, 402, 408, 416, 425, 427, 444, 486, 490, 494, or in a corresponding position in a homologous glucoamylase which displays at least 60% homology with the amino acid sequences shown in SEQ ID NO: 2.

Detail Description Paragraph - DETX (76):
[0108] Examples of suitable promoters for directing the transcription of the DNA sequence encoding a glucoamylase variant of the invention, especially in a bacterial host, are the promoter of the lac operon of E.coli, the Streptomyces coelicolor agarase gene dagA promoters, the promoters of the Bacillus licheniformis alpha-amylase gene (amyL), the promoters of the Bacillus stearothermophilus maltogenic amylase gene (amyM), the promoters of the Bacillus amyloliquefaciens alpha.-amylase (amyQ), the promoters of the Bacillus subtilis xylA and xylB genes etc. For transcription in a fungal host, examples of useful promoters are those derived from the gene encoding A. oryzae TAKA amylase, the TPI (triose phosphate isomerase) promoter from S. cerevisiae (Alber et al. (1982), J. Mol. Appl. Genet 1, p. 419-434, Rhizomucor miehei aspartic proteinase, A. niger neutral alpha-amylase, A. niger acid stable alpha-amylase, A. niger glucoamylase, Rhizomucor miehei lipase, A. oryzae alkaline protease, A. oryzae triose phosphate isomerase or A. nidulans acetamidase.

Detail Description Paragraph - DETX (102):
[0134] Further, by improving the thermal stability the T.sub.1/2 (half-time, as defined in the "Materials and Methods" section) is improved. As the thermal stability of the glucoamylase variants of the invention is improved a minor amount of glucoamylase need to be added to replace the glucoamylase being inactivated during the saccharification process. More glucoamylase is maintained active during saccharification process according to the present invention. Furthermore, the risk of microbial contamination is also reduced when carrying the saccharification process at temperature above 63.degree. C.

Detail Description Paragraph - DETX (148):

[0180] The thermal stability of variants is determined as T.sub.1/2 using the following method: 950 microliter 50 mM sodium acetate buffer (pH 4.3) (NaOAc) is incubated for 5 minutes at 68.degree. C., 70.degree. C. or 75.degree. C. 50 microliter enzyme in buffer (4 AGU/ml) is added. 2.times.40 microliter samples are taken at, e.g., 0, 5, 10, 20, 30 and 40 minutes and chilled on ice. The activity (AGU/ml) measured before incubation (0 minutes) is used as reference (100%). The decline in stability (in percent) is calculated as a function of the incubation time. The % residual glucoamylase activity is determined at different times. T.sub.1/2 is the period of time until which the % relative activity is decreased to 50%.

Detail Description Paragraph - DETX (175):

[0207] 3. Decide on which kind of mutations should be carried out, e.g., with respect to the desired stability and/or performance of the variant to be constructed,

Claims Text - CLTX (10):

10. The variant of any of claims 1-9, wherein the variant has improved thermal stability when compared with the parent glucoamylase.

PGPUB-DOCUMENT-NUMBER: 20030224964

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030224964 A1

TITLE: Laundry detergent compositions comprising zwitterionic polyamines

PUBLICATION-DATE: December 4, 2003

INVENTOR-INFORMATION:

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Price, Kenneth Nathan	Wyoming	OH	US	

APPL-NO: 10/ 232357

DATE FILED: August 30, 2002

RELATED-US-APPL-DATA:

child 10232357 A1 20020830

parent continuation-of 10129618 20020508 US PENDING

US-CL-CURRENT: 510/499

ABSTRACT:

The present invention relates to laundry detergent compositions comprising:
A) from about 0.01%, preferably from about 0.1%, more preferably from about 1%, most preferably from about 3% to about 50%, preferably to about 20%, more preferably to about 10%, most preferably to about 7% by weight, of a hydrophobically modified polyamine having the formula: 1
wherein R is C.sub.5-C.sub.20 linear or branched alkylene, and mixtures thereof; R.sup.1 is an alkyleneoxy unit having the formula:
--(R.sup.2O).sub.x--R.sup.3
wherein R.sup.2 is C.sub.2-C.sub.4 linear or branched alkylene, and mixtures thereof; at least one R.sup.3 is an anionic unit, and the remaining R.sup.3 moieties are selected from the group consisting of hydrogen, C.sub.1-C.sub.22 alkyl, C.sub.7-C.sub.22 alkylenearyl, an anionic unit, and mixtures thereof; x is from about 15 to about 30; Q is a hydrophobic quaternizing unit selected from the group consisting of C.sub.8-C.sub.30 linear or branched alkyl, C.sub.6-C.sub.30 cycloalkyl, C.sub.7-C.sub.30 substituted or unsubstituted alkylenearyl, and mixtures thereof; X is an anion present in sufficient amount to provide electronic neutrality; n is from 0 to 4;
B) from about 0.01% by weight, of a surfactant system comprising one or more surfactants selected from:
i) from 0% to 100% by weight, of one or more anionic surfactants;
ii) from 0% to 100% by weight, of one or more nonionic surfactants;
iii) optionally from 0.1% to about 80% by weight, of one or more cationic surfactants;
iv) optionally from 0.1% to about 80% by weight, of one or more zwitterionic surfactants;
v) optionally from 0.1% to about 80% by weight, of one or more ampholytic surfactants; or
vi) mixtures thereof;

C) the balance carriers and adjunct ingredients.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This Application claims the benefit of U.S. application Ser. No. 10/129,618 filed on May 8, 2002, which in turn claims the benefit of U.S. Provisional Application Serial No. 60/164,283 filed on Nov. 9, 1999, (now abandoned); and in addition, this Application claims the benefit of U.S. application Ser. No. 09/790,042 filed on Feb. 21, 2001, which in turn claims the benefit of U.S. Provisional Application No. 60/184,250, filed on Feb. 23, 2000, (now abandoned).

---------- KWIC ---------

Pre-Grant Publication Document Identifier - DID (1):
US 20030224964 A1

Summary of Invention Paragraph - BSTX (260):
[0247] A preferred protease enzyme for use in the present invention is a variant of Protease A (BPN') which is a non-naturally occurring carbonyl hydrolase variant having a different proteolytic activity, stability, substrate specificity, pH profile and/or performance characteristic as compared to the precursor carbonyl hydrolase from which the amino acid sequence of the variant is derived. This variant of BPN' is disclosed in EP 130,756 A, Jan. 9, 1985. Specifically Protease A-BSV is BPN' wherein the Gly at position 166 is replaced with Asn, Ser, Lys, Arg, His, Gin, Ala, or Glu; the Gly at position 169 is replaced with Ser; the Met at position 222 is replaced with Gln, Phe, Cys, His, Asn, Glu, Ala or Thr; or alternatively the Gly at position 166 is replaced with Lys, and the Met at position 222 is replaced with Cys; or alternatively the Gly at position 169 is replaced with Ala and the Met at position 222 is replaced with Ala.

Summary of Invention Paragraph - BSTX (262):
[0249] A preferred protease enzyme for use in the present invention is Protease B. Protease B is a non-naturally occurring carbonyl hydrolase variant having a different proteolytic activity, stability, substrate specificity, pH profile and/or performance characteristic as compared to the precursor carbonyl hydrolase from which the amino acid sequence of the variant is derived. Protease B is a variant of BPN' in which tyrosine is replaced with leucine at position +217 and as further disclosed in EP 303,761 A, Apr. 28, 1987 and EP 130,756 A, Jan. 9, 1985.

Summary of Invention Paragraph - BSTX (286):
[0272] Amylases suitable herein include, for example, .alpha.-amylases described in GB 1,296,839 to Novo; RAPIDASE.RTM., International Bio-Synthetics, Inc. and TERMAMYL.RTM., Novo. FUNGAMYL.RTM. from Novo is especially useful. Engineering of enzymes for improved stability, e.g., oxidative stability, is known. See, for example J. Biological Chem., Vol. 260, No. 11, June 1985, pp 6518-6521 and WO 9402597 to Novo, Feb. 3, 1994, and WO 9509909 A to Novo. Certain preferred embodiments of the present compositions can make use of amylases having improved stability in detergents, especially improved oxidative stability as measured against a reference-point of TERMAMYL.RTM. in commercial use in 1993. These preferred amylases herein share the characteristic of being "stability-enhanced" amylases, characterized, at a minimum, by a measurable improvement in one or more of: oxidative stability, e.g., to hydrogen peroxide/tetraacetylethylenediamine in buffered solution at pH 9-10; thermal stability, e.g., at common wash temperatures such as about 60.degree. C.; or

alkaline stability, e.g., at a pH from about 8 to about 11, measured versus the above-identified reference-point amylase. Stability can be measured using any of the art-disclosed technical tests. See, for example, references disclosed in WO 9402597. Stability-enhanced amylases can be obtained from Novo or from Genencor International. One class of highly preferred amylases herein have the commonality of being derived using site-directed mutagenesis from one or more of the Baccillus amylases, especially the Bacillus .alpha.-amylases, regardless of whether one, two or multiple amylase strains are the immediate precursors. Oxidative stability-enhanced amylases vs. the above-identified reference amylase are preferred for use, especially in bleaching, more preferably oxygen bleaching, as distinct from chlorine bleaching, detergent compositions herein. Such preferred amylases include (a) an amylase according to the hereinbefore incorporated WO 9402597, Novo, Feb. 3, 1994, as further illustrated by a mutant in which substitution is made, using alanine or threonine, preferably threonine, of the methionine residue located in position 197 of the B. licheniformis alpha-amylase, known as TERMAMYL.RTM., or the homologous position variation of a similar parent amylase, such as B. amyloliquefaciens, B. subtilis, or B. stearothermophilus; (b) stability-enhanced amylases as described by Genencor International in a paper entitled "Oxidatively Resistant alpha-Amylases" presented at the 207th American Chemical Society National Meeting, Mar. 13-17, 1994, by C. Mitchinson. Therein it was noted that bleaches in automatic dishwashing detergents inactivate alpha-amylases but that improved oxidative stability amylases have been made by Genencor from B. licheniformis NCIB8061. Methionine (Met) was identified as the most likely residue to be modified. Met was substituted, one at a time, in positions 8, 15, 197, 256, 304, 366 and 438 leading to specific mutants, particularly important being M197L and M197T with the M197T variant being the most stable expressed variant. Stability was measured in CASCADE.RTM.) and SUNLIGHT.RTM.; (c) particularly preferred amylases herein include amylase variants having additional modification in the immediate parent as described in WO 9510603 A and are available from the assignee, Novo, as DURAMYL.RTM.. Other particularly preferred oxidative stability enhanced amylase include those described in WO 9418314 to Genencor International and WO 9402597 to Novo. Any other oxidative stability-enhanced amylase can be used, for example as derived by site-directed mutagenesis from known chimeric, hybrid or simple mutant parent forms of available amylases. Other preferred enzyme modifications are accessible. See WO 9509909 A to Novo.

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PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030215928 A1

TITLE: Amylolytic enzyme variants

PUBLICATION-DATE: November 20, 2003

INVENTOR-INFORMATION:

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APPL-NO: 10/ 453828

DATE FILED: May 29, 2003

RELATED-US-APPL-DATA:

child 10453828 A1 20030529

parent division-of 10234266 20020904 US PENDING

child 10234266 20020904 US

parent division-of 09645707 20000824 US GRANTED

parent-patent 6482622 US

child 09645707 20000824 US

parent continuation-of PCT/DK99/00087 19990226 US UNKNOWN

non-provisional-of-provisional 60077509 19980311 US

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COUNTRY	APPL-NO	DOC-ID	APPL-DATE
DK	1998 00269	1998DK-1998 00269	February 27, 1998
DK	1998 00273	1998DK-1998 00273	February 27, 1998

US-CL-CURRENT: 435/97, 435/193 , 435/252.3 , 435/252.31 , 435/320.1 , 435/69.1 , 536/23.2

ABSTRACT:

The inventors have discovered some striking, and not previously predicted structural similarities and differences between the structure of Novamyl and the reported structures of CGTases, and based on this they have constructed variants of maltogenic alpha-amylase having CGTase activity and variants of CGTase having maltogenic alpha-amylase activity. Further, on the basis of

sequence homology between Novamyl and CGTases, the inventors have constructed hybrid enzymes with one or more improvements to specific properties of the parent enzymes, using recombinant DNA methodology.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a divisional of U.S. Ser. No. 10/234,266, filed on Sep. 4, 2002, which is a divisional of U.S. Ser. No. 09/645,707, filed on Aug. 24, 2000 (now U.S. Pat. No. 6,482,622), which is a continuation of PCT/DK/99/00087, filed on Feb. 26, 1999, and claims priority under 35 U.S.C. 119 of Danish application nos. PA 1998 00269 and PA 1998 00273, both filed on Feb. 27, 1998, and U.S. provisional application Nos. 60/077,509 and 60/077,795, filed on Mar. 11, 1998 and Mar. 12, 1998, respectively, the contents of which are fully incorporated herein by reference.

---------- KWIC ---------

Pre-Grant Publication Document Identifier - DID (1):
US 20030215928 A1

Detail Description Paragraph - DETX (94):
[0122] In the vector, the DNA sequence should be operably connected to a suitable promoter sequence. The promoter may be any DNA sequence which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. Examples of suitable promoters for directing the transcription of the DNA sequence encoding a maltogenic alpha-amylase variant of the invention, especially in a bacterial host, are the promoter of the lac operon of E. coli, the Streptomyces coelicolor agarase gene dagA promoters, the promoters of the Bacillus licheniformis .alpha.-amylase gene (amyL), the promoters of the Bacillus stearothermophilus maltogenic amylase gene (amyM), the promoters of the Bacillus amyloliquefaciens .alpha.-amylase (amyQ), the promoters of the Bacillus subtilis xylA and xylB genes, etc. For transcription in a fungal host, examples of useful promoters are those derived from the gene encoding A. oryzae TAKA amylase, Rhizomucor miehei aspartic proteinase, A. niger neutral .alpha.-amylase, A. niger acid stable .alpha.-amylase, A. niger glu-coamylase, Rhizomucor miehei lipase, A. oryzae alkaline protease, A. oryzae triose phosphate isomerase or A. nidulans acetamidase.

Detail Description Paragraph - DETX (109):
[0137] To screen for variants with increased stability, the filter with bound maltogenic alpha-amylase variants can be pretreated prior to the detection step described above to inactivate variants that do not have improved stability relative to the parent CGTase. This inactivation step may consist of, but is not limited to, incubation at elevated temperatures in the presence of a buffered solution at any pH from pH 2 to 12, and/or in a buffer containing another compound known or thought to contribute to altered stability e.g., surfactants, EDTA, EGTA, wheat flour components, or any other relevant additives. Filters so treated for a specified time are then rinsed briefly in deionized water and placed on plates for activity detection as described above. The conditions are chosen such that stabilized variants show increased enzymatic activity relative to the parent after incubation on the detection media.

Detail Description Paragraph - DETX (110):
[0138] To screen for variants with altered thermostability, filters with bound variants are incubated in buffer at a given pH (e.g., in the range from

pH 2-12) at an elevated temperature (e.g., in the range from 50.degree.-110.degree. C.) for a time period (e.g., from 1-20 minutes) to inactivate nearly all of the parent CGTase, rinsed in water, then placed directly on a detection plate containing immobilized Cibacron Red labeled amylopectin and incubated until activity is detectable. Similarly, pH dependent stability can be screened for by adjusting the pH of the buffer in the above inactivation step such that the parent CGTase is inactivated, thereby allowing detection of only those variants with increased stability at the pH in question. To screen for variants with increased calcium-dependent stability calcium chelators, such as ethylene glycol-bis(.beta.-aminoethyl ether) N,N,N',N'-tetraacetic acid (EGTA), is added to the inactivation buffer at a concentration such that the parent CGTase is inactivated under conditions further defined, such as buffer pH, temperature or a specified length of incubation.

Detail Description Paragraph - DETX (111):

[0139] The variants of the invention may be suitably tested by assaying the starch-degrading activity of the variant, for instance by growing host cells transformed with a DNA sequence encoding a variant on a starch-containing agarose plate and identifying starch-degrading host cells as described above. Further testing in regard to altered properties, including specific activity, substrate specificity, cleavage pattern, thermoactivation, thermostability, pH dependent activity or optimum, pH dependent stability, temperature dependent activity or optimum, transglycosylation activity, stability, and any other parameter of interest, may be performed on purified variants in accordance with methods known in the art as described below.

Detail Description Paragraph - DETX (164):

[0184] In this example, the unique active site loop was used to select hybrid enzymes with maltogenic alpha-amylase activity from a library of random recombinants. In this method, Novamyl and the cyclic maltodextrin glucosyl transferase (CGTase) from Bacillus circulans, were randomly recombined by the DNA shuffling method of Crameri A, et al., op.cit. Those resulting mutants containing the Novamyl loop were selected using PCR as described above in Example 2.

PGPUB-DOCUMENT-NUMBER: 20030211958

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030211958 A1

TITLE: Alpha-amylase mutants

PUBLICATION-DATE: November 13, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Svendsen, Allan	Birkerod		DK	
Borchert, Torben Vedel	Copenhagen		DK	
Bisgard-Frantzen, Henrik	Bagsvaerd		DK	
Outtrup, Helle	Ballerup		DK	
Nielsen, Bjarne Ronfeldt	Virum		DK	
Nielsen, Vibeke Skovgaard	Bagsvaerd		DK	
Hedegaard, Lisbeth	Skodsborg		DK	

APPL-NO: 10/ 327837

DATE FILED: December 23, 2002

RELATED-US-APPL-DATA:

child 10327837 A1 20021223

parent division-of 09545586 20000407 US GRANTED

parent-patent 6528298 US

child 09545586 20000407 US

parent division-of 09290734 19990413 US GRANTED

parent-patent 6361989 US

child 09290734 19990413 US

parent continuation-in-part-of 09170670 19981013 US GRANTED

parent-patent 6187576 US

non-provisional-of-provisional 60063306 19971028 US

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
DK	1172/97	1997DK-1172/97	October 13, 1997
DK	1999 00439	1999DK-1999 00439	March 31, 1999

US-CL-CURRENT: 510/226, 435/202 , 435/320.1 , 435/325 , 435/69.1 , 510/320 , 536/23.2

ABSTRACT:

The invention relates to a novel Termamyl-like alpha-amylase, and Termamyl-like alpha-amylases comprising mutations in two, three, four, five or six

regions/positions. The variants have increased thermostability at acidic pH and/or at low Ca.sup.2+ concentrations (relative to the parent). The invention also relates to a DNA construct comprising a DNA sequence encoding an alpha-amylase variant of the invention, a recombinant expression vector which carries a DNA construct of the invention, a cell which is transformed with a DNA construct of the invention, the use of an alpha-amylase variant of the invention for washing and/or dishwashing, textile desizing, starch liquefaction, a detergent additive comprising an alpha-amylase variant of the invention, a manual or automatic dishwashing detergent composition comprising an alpha-amylase variant of the invention, a method for generating a variant of a parent Termamyl-like alpha-amylase, which variant exhibits increased thermostability at acidic pH and/or at low Ca.sup.2+ concentrations (relative to the parent).

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a divisional of application Ser. No. 09/545,586, filed on Apr. 7, 2000 (now allowed), which is a divisional of application Ser. No. 09/290,734 filed on Apr. 13, 1999 (now U.S. Pat. No. 6,361,981), which is a continuation-in-part of application Ser. No. 09/170,670 filed on Oct. 13, 1998 (now U.S. Pat. No. 6,187,576), and claims priority under 35 U.S.C. 119 of Danish application no. 1172/97, filed on Oct. 13, 1997, and Danish application no. PA 1999 00439, filed on Mar. 31, 1999, and U.S. application Ser. No. 60/063,306, filed on Oct. 28, 1997, and, the contents of which are fully incorporated herein by reference.

---------- KWIC ---------

Pre-Grant Publication Document Identifier - DID (1):
US 20030211958 A1

Summary of Invention Paragraph - BSTX (6):
[0005] Among more recent disclosures relating to alpha-amylases, WO 96/23874 provides three-dimensional, X-ray crystal structural data for a Termamyl-like alpha-amylase which consists of the 300 N-terminal amino acid residues of the B. amyloliquefaciens alpha-amylase and amino acids 301-483 of the C-terminal end of the B. licheniformis alpha-amylase comprising the amino acid sequence (the latter being available commercially under the tradename Termamyl.TM.), and which is thus closely related to the industrially important Bacillus alpha-amylases (which in the present context are embraced within the meaning of the term "Termamyl-like alpha-amylases", and which include, inter alia, the B. licheniformis, B. amyloliquefaciens and B. stearothermophilus alpha-amylases). WO 96/23874 further describes methodology for designing, on the basis of an analysis of the structure of a parent Termamyl-like alpha-amylase, variants of the parent Termamyl-like alpha-amylase which exhibit altered properties relative to the parent.

Detail Description Paragraph - DETX (172):
[0198] In relation to the above, a further aspect of the present invention relates to a method for generating a variant of a parent alpha-amylase, e.g. wherein the variant exhibits altered or increased thermal stability relative to the parent, the method comprising:

Detail Description Paragraph - DETX (175):
[0201] (c) screening for host cells expressing analpha-amylase variant which has an altered property (i.e. thermal stability) relative to the parent alpha-amylase.

Detail Description Paragraph - DETX (195):

[0221] In the vector, the DNA sequence should be operably connected to a suitable promoter sequence. The promoter may be any DNA sequence which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. Examples of suitable promoters for directing the transcription of the DNA sequence encoding an alpha-amylase variant of the invention, especially in a bacterial host, are the promoter of the lac operon of E. coli, the Streptomyces coelicolor agarase gene dagA promoters, the promoters of the Bacillus licheniformis alpha-amylase gene (amyL), the promoters of the Bacillus stearothermophilus maltogenic amylase gene (amyM), the promoters of the Bacillus amyloliquefaciens alpha-amylase (amyQ), the promoters of the Bacillus subtilis xylA and xylB genes etc. For transcription in a fungal host, examples of useful promoters are those derived 25 from the gene encoding A. oryzae TAKA amylase, Rhizomucor miehei aspartic proteinase, A. niger neutral alpha-amylase, A. niger acid stable alpha-amylase, A. niger glucoamylase, Rhizomucor miehei lipase, A. oryzae alkaline protease, A. oryzae triose phosphate isomerase or A. nidulans acetamidase.

Detail Description Paragraph - DETX (229):

[0255] Amylases: Suitable amylases (alpha- and/or -) include those of bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Amylases include, for example, alpha-amylases obtained from Bacillus, e.g., a special strain of B. licheniformis, described in more detail in GB 1,296,839.

Claims Text - CLTX (30):

30. The variant according to claim 21, exhibiting increased stability at acidic pH and/or low Ca.sup.2+ concentration.

Claims Text - CLTX (56):

56. A method for generating a variant of a parent Termamyl-like alpha-amylase of claims 21-31, which variant exhibits increased stability at low pH and at low calcium concentration relative to the parent, the method comprising: (a) subjecting a DNA sequence encoding the parent Termamyl-like alpha-amylase to random mutagenesis, (b) expressing the mutated DNA sequence obtained in step (a) in a host cell, and (c) screening for host cells expressing a mutated alpha-amylase which has increased stability at low pH and low calcium concentration relative to the parent alpha-amylase.

PGPUB-DOCUMENT-NUMBER: 20030207408

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030207408 A1

TITLE: Amylolytic enzyme variants

PUBLICATION-DATE: November 6, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Cherry, Joel Robert	Davis	CA	US	
Svendsen, Allan	Birkerod		DK	
Andersen, Carsten	Vaerloese		DK	
Beier, Lars	Lyngby		DK	
Frandsen, Torben Peter	Frederiksberg C		DK	
Schafer, Thomas	Farum		DK	

APPL-NO: 10/ 442558

DATE FILED: May 21, 2003

RELATED-US-APPL-DATA:

child 10442558 A1 20030521

parent division-of 10234266 20020904 US PENDING

child 10234266 20020904 US

parent division-of 09645707 20000824 US GRANTED

parent-patent 6482622 US

child 09645707 20000824 US

parent continuation-of PCT/DK99/00087 19990226 US UNKNOWN

non-provisional-of-provisional 60077509 19980311 US

non-provisional-of-provisional 60077795 19980312 US

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
DK	1998 00269	1998DK-1998 00269	February 27, 1998
DK	1998 00273	1998DK-1998 00273	February 27, 1998

US-CL-CURRENT: 435/101, 426/20

ABSTRACT:

The inventors have discovered some striking, and not previously predicted structural similarities and differences between the structure of Novamyl and the reported structures of CGTases, and based on this they have constructed variants of maltogenic alpha-amylase having CGTase activity and variants of CGTase having maltogenic alpha-amylase activity. Further, on the basis of sequence homology between Novamyl.RTM. and CGTases, the inventors have

constructed hybrid enzymes with one or more improvements to specific properties of the parent enzymes, using recombinant DNA methodology.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a divisional of U.S. Ser. No. 10/234,266, filed on Sep. 4, 2002, which is a divisional of U.S. Ser. No. 09/645,707, filed on Aug. 24, 2000 (now U.S. Pat. No. 6,482,622), which is a continuation of PCT/DK/99/00087, filed on Feb. 26, 1999, and claims priority under 35 U.S.C. 119 of Danish application nos. PA 1998 00269 and PA 1998 00273, both filed on Feb. 27, 1998, and U.S. provisional application Nos. 60/077,509 and 60/077,795, filed on Mar. 11, 1998 and Mar. 12, 1998, respectively, the contents of which are fully incorporated herein by reference.

---------- KWIC ---------

Pre-Grant Publication Document Identifier - DID (1):
US 20030207408 A1

Detail Description Paragraph - DETX (94):
[0123] In the vector, the DNA sequence should be operably connected to a suitable promoter sequence. The promoter may be any DNA sequence which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. Examples of suitable promoters for directing the transcription of the DNA sequence encoding a maltogenic alpha-amylase variant of the invention, especially in a bacterial host, are the promoter of the lac operon of E. coli, the Streptomyces coelicolor agarase gene dagA promoters, the promoters of the Bacillus licheniformis .alpha.-amylase gene (amyL), the promoters of the Bacillus stearothermophilus maltogenic amylase gene (amyM), the promoters of the Bacillus amyloliquefaciens .alpha.-amylase (amyQ), the promoters of the Bacillus subtilis xylA and xylB genes, etc. For transcription in a fungal host, examples of useful promoters are those derived from the gene encoding A. oryzae TAKA amylase, Rhizomucor miehei aspartic proteinase, A. niger neutral .alpha.-amylase, A. niger acid stable .alpha.-amylase, A. niger glucoamylase, Rhizomucor miehei lipase, A. oryzae alkaline protease, A. oryzae triose phosphate isomerase or A. nidulans acetamidase.

Detail Description Paragraph - DETX (110):
[0139] To screen for variants with increased stability, the filter with bound maltogenic alpha-amylase variants can be pretreated prior to the detection step described above to inactivate variants that do not have improved stability relative to the parent CGTase. This inactivation step may consist of, but is not limited to, incubation at elevated temperatures in the presence of a buffered solution at any pH from pH 2 to 12, and/or in a buffer containing another compound known or thought to contribute to altered stability e.g., surfactants, EDTA, EGTA, wheat flour components, or any other relevant additives. Filters so treated for a specified time are then rinsed briefly in deionized water and placed on plates for activity detection as described above. The conditions are chosen such that stabilized variants show increased enzymatic activity relative to the parent after incubation on the detection media.

Detail Description Paragraph - DETX (111):
[0140] To screen for variants with altered thermostability, filters with bound variants are incubated in buffer at a given pH (e.g., in the range from pH 2-12) at an elevated temperature (e.g., in the range from 500-110.degree.

C.) for a time period (e.g., from 1-20 minutes) to inactivate nearly all of the parent CGTase, rinsed in water, then placed directly on a detection plate containing immobilized Cibacron Red labeled amylopectin and incubated until activity is detectable. Similarly, pH dependent stability can be screened for by adjusting the pH of the buffer in the above inactivation step such that the parent CGTase is inactivated, thereby allowing detection of only those variants with increased stability at the pH in question. To screen for variants with increased calcium-dependent stability calcium chelators, such as ethylene glycol-bis(.beta.-aminoethyl ether) N,N,N',N'-tetraacetic acid (EGTA), is added to the inactivation buffer at a concentration such that the parent CGTase is inactivated under conditions further defined, such as buffer pH, temperature or a specified length of incubation.

Detail Description Paragraph - DETX (112):

[0141] The variants of the invention may be suitably tested by assaying the starch-degrading activity of the variant, for instance by growing host cells transformed with a DNA sequence encoding a variant on a starch-containing agarose plate and identifying starch-degrading host cells as described above. Further testing in regard to altered properties, including specific activity, substrate specificity, cleavage pattern, thermoactivation, thermostability, pH dependent activity or optimum, pH dependent stability, temperature dependent activity or optimum, transglycosylation activity, stability, and any other parameter of interest, may be performed on purified variants in accordance with methods known in the art as described below.

Detail Description Paragraph - DETX (165):

[0187] In this example, the unique active site loop was used to select hybrid enzymes with maltogenic alpha-amylase activity from a library of random recombinants. In this method, Novamyl and the cyclic maltodextrin glucosyl transferase (CGTase) from Bacillus circulans, were randomly recombined by the DNA shuffling method of Crameri A, et al., op.cit. Those resulting mutants containing the Novamyl loop were selected using PCR as described above in Example 2.

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PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030190738 A1

TITLE: Starch debranching enzymes

PUBLICATION-DATE: October 9, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Bisgard-Frantzen, Henrik	Bagsvaerd		DK	
Svendsen, Allan	Birkerod		DK	

APPL-NO: 10/ 375720

DATE FILED: February 26, 2003

RELATED-US-APPL-DATA:

child 10375720 A1 20030226

parent continuation-of 09833435 20010412 US PENDING

child 09833435 20010412 US

parent continuation-of 09346237 19990701 US GRANTED

parent-patent 6265197 US

non-provisional-of-provisional 60094353 19980728 US

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
DK	1998 00868	1998DK-1998 00868	July 2, 1998

US-CL-CURRENT: 435/210, 435/101 , 435/105 , 435/320.1 , 435/325 , 435/69.1

ABSTRACT:

The invention relates to a genetically engineered variant of a parent starch debranching enzyme, i.e. a pullulanase or an isamylase, the enzyme variant having an improved thermostability at a pH in the range of 4-6 compared to the parent enzyme and/or an increased activity towards amylopectin and/or glycogen compared to the parent enzyme, to methods for producing such starch debranching enzyme variants with improved thermostability and/or altered substrate specificity, and to a method for converting starch to one or more sugars using at least one such enzyme variant.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation of U.S. application Ser. No. 09/833,435, filed on Mar. 26, 2001 (abandoned), which is a continuation of U.S. application Ser. No. 09/346,237, filed Jul. 1, 1999 (now U.S. Pat. No. 6,265,197), which claims priority under 35 U.S.C. 119 of Danish application PA 1998 00868, filed Jul. 2, 1998, and the benefit of U.S. provisional application No. 60/094,353, filed on Jul. 28, 1998, the contents

of which are fully incorporated herein by reference.

---------- KWIC ---------

Pre-Grant Publication Document Identifier - DID (1):
US 20030190738 A1

Summary of Invention Paragraph - BSTX (75):
[0072] Examples of specific .alpha.-amylases which can be used in the liquefaction step include Bacillus licheniformis .alpha.-amylases, such as the commercially available products Termamyl.RTM., Spezyme.RTM. AA, Spezyme.RTM. Delta AA, Maxamyl.RTM. and Kleistase.RTM., and the .alpha.-amylase mutants described in WO 96/23874 (Novo Nordisk) and PCT/DK97/00197 (Novo Nordisk).

Summary of Invention Paragraph - BSTX (195):
[0192] In relation to the above, a further aspect of the present invention relates to a method for generating a variant of a parent enzyme, wherein the variant exhibits improved thermal stability relative to the parent, the method comprising:

Summary of Invention Paragraph - BSTX (198):
[0195] (c) screening for host cells expressing an enzyme variant which has an altered property (e.g. thermal stability) relative to the parent enzyme.

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PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030171236 A1

TITLE: ALPHA-AMYLASE MUTANTS

PUBLICATION-DATE: September 11, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Svendsen, Allan	Birkerod		DK	
Borchert, Torben Vedel	Jyllinge		DK	
Bisgard-Frantzen, Henrik	Bagsvaerd		DK	

APPL-NO: 10/ 186042

DATE FILED: June 28, 2002

RELATED-US-APPL-DATA:

child 10186042 A1 20020628

parent division-of 09672459 20000928 US GRANTED

parent-patent 6436888 US

child 09672459 20000928 US

parent continuation-of 09182859 19981029 US GRANTED

parent-patent 6143708 US

child 09182859 19981029 US

parent continuation-of PCT/DK97/00197 19970430 US UNKNOWN

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
DK	0515/96	1996DK-0515/96	April 30, 1996
DK	0712/96	1996DK-0712/96	June 28, 1996
DK	0775/96	1996DK-0775/96	July 11, 1996
DK	1263/96	1996DK-1263/96	November 8, 1996

US-CL-CURRENT: 510/305, 435/202 , 510/226

ABSTRACT:

The invention relates to a variant of a parent Termamyl-like a-amylase, which variant has a-amylase activity and exhibits an alteration in at least one of the following properties relative to said parent a-amylase: substrate specificity, substrate binding, substrate cleavage pattern, thermal stability, pH/activity profile, pH/stability profile, stability towards oxidation, Ca.sup.2+ dependency and specific activity.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a division of Ser. No. 09/672,459, filed on Sep. 28, 2000, which is a continuation of Ser. No. 09/182,859, filed on Oct. 29, 1998 (now U.S. Pat. No. 6,143,708), which is a continuation of PCT/DK97/00197 filed Apr. 30, 1997 which claims priority under 35 U.S.C. 119 of Danish applications 0515/96 filed Apr. 30, 1996, 0712/96 filed Jun. 28, 1996, 0775/96 filed Jul. 11, 1996, and 1263/96 filed Nov. 8, 1996, the contents of which are fully incorporated herein by reference.

---------- KWIC ---------

Pre-Grant Publication Document Identifier - DID (1):
US 20030171236 A1

Summary of Invention Paragraph - BSTX (5):
[0004] Among more recent disclosures relating to .alpha.-amylases, WO 96/23874 provides three-dimensional, X-ray crystal structural data for a Termamyl-like a-amylase which consists of the 300 N-terminal amino acid residues of the B. amyloliquefaciens .alpha.-amylase comprising the amino acid sequence shown in SEQ ID No. 4 herein and amino acids 301-483 of the C-terminal end of the B. licheniformis .alpha.-amylase comprising the amino acid sequence shown in SEQ ID No. 2 herein (the latter being available commercially under the tradename Termamyl.TM.), and which is thus closely related to the industrially important Bacillus .alpha.-amylases (which in the present context are embraced within the meaning of the term "Termamyl-like .alpha.-amylases", and which include, inter alia, the B. licheniformis, B. amyloliquefaciens and B. stearothermophilus .alpha.-amylases). WO 96/23874 further describes methodology for designing, on the basis of an analysis of the structure of a parent Termamyl-like .alpha.-amylase, variants of the parent Termamyl-like .alpha.-amylase which exhibit altered properties relative to the parent.

Summary of Invention Paragraph - BSTX (177):
[0174] Furthermore, it is preferred that the mutagenesis is carried out by use of doped or spiked oligonucleotides. The doping is preferably done so as to introduce amino acids contributing to improved stability at low pH and reduced calcium dependency at low pH of the resulting .alpha.-amylase variant. Furthermore, when selecting the doping scheme, the possibility of introducing Asn and Gln residues should generally be avoided, since Asn and Gln residues in general are associated with instability at low pH. Preferably, when a Pro residue can be inserted with potential benefits (e.g. as assessed from protein-structural considerations), the doping scheme is prepared to include a preference for introduction of a Pro residue.

Summary of Invention Paragraph - BSTX (179):
[0176] In relation to the above, a further aspect of the present invention relates to a method for generating a variant of a parent Termamyl-like .alpha.-amylase, which variant exhibits increased stability at low pH and at low calcium concentration relative to the parent, the method comprising:

Summary of Invention Paragraph - BSTX (207):
[0204] .alpha.-Amylase activity is detected by Cibacron Red labelled amylopectin, which is immobilized on agarose. For screening for variants with increased thermal and high-pH stability, the filter with bound .alpha.-amylase variants is incubated in a buffer at pH 10.5 and 600 or 65.degree. C. for a specified time, rinsed briefly in deionized water and placed on the amylopectin-agarose matrix for activity detection. Residual activity is seen as lysis of Cibacron Red by amylopectin degradation. The conditions are chosen to be such that activity due to the .alpha.-amylase having the amino acid

sequence shown in SEQ ID No. 2 can barely be detected. Stabilized variants show, under the same conditions, increased colour intensity due to increased liberation of Cibacron Red.

Summary of Invention Paragraph - BSTX (214):
[0211] In the vector, the DNA sequence should be operably connected to a suitable promoter sequence. The promoter may be any DNA sequence which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. Examples of suitable promoters for directing the transcription of the DNA sequence encoding an .alpha.-amylase variant of the invention, especially in a bacterial host, are the promoter of the lac operon of E. coli, the Streptomyces coelicolor agarase gene dagA promoters, the promoters of the Bacillus licheniformis .alpha.-amylase gene (amyL), the promoters of the Bacillus stearothermophilus maltogenic amylase gene (amyM), the promoters of the Bacillus amyloliquefaciens .alpha.-amylase (amyQ), the promoters of the Bacillus subtilis xylA and xylB genes etc. For transcription in a fungal host, examples of useful promoters are those derived from the gene encoding A. oryzae TAKA amylase, Rhizomucor miehei aspartic proteinase, A. niger neutral .alpha.-amylase, A. niger acid stable .alpha.-amylase, A. niger glucoamylase, Rhizomucor miehei lipase, A. oryzae alkaline protease, A. oryzae triose phosphate isomerase or A. nidulans acetamidase.

Detail Description Paragraph - DETX (17):
[0324] 3. Decide on which kind of mutations should be carried out, e.g. with respect to the desired stability and/or performance of the variant to be constructed

Detail Description Paragraph - DETX (92):
[0397] Construction, by Localized Random, Doped Mutagenesis, of Termamyl-Like .alpha.-Amylase Variants Having an Improved Stability at Low pH and a Reduced Dependency on Calcium Ions for Stability Compared to the Parent Enzyme

Detail Description Paragraph - DETX (95):
[0400] has a very satisfactory stability at low pH and low calcium concentrations. In an attempt to further improve the stability at low pH and low calcium concentration of said .alpha.-amylase variant random mutagenesis in preselected regions wase performed.

Detail Description Paragraph - DETX (258):
[0563] The mutations indicated in bold were introduced by the random mutagenesis method. The stability data for these variants appear from Table 11 in Example 3.

Detail Description Paragraph - DETX (284):
[0587] This example summarises the stability results of variants characterised by a fluorimetric assay at 70.degree. C. under two different conditions, (1) pH 4.5 and 1 mM CaCl.sub.2 and (2) pH 6.2 and 10 .mu.M CaCl.sub.2.

Claims Text - CLTX (2):
2. A variant according to claim 1, exhibiting increased stability at low pH and low Ca.sup.2+ concentration relative to the parent Termamyl-like .alpha.-amylase, and comprising mutations selected from the following: H156Y+A181T+A209V; H156Y+A181T+N190F+A209V+Q264S; A1*+N2*+L3V+M15T+R23K+S-29A+A30E+Y31H+A33S+E34D+H35I+H156Y+A181T+A209V; A1*+N2*+L3V+M15T+R23K+S29A-+A30E+Y31H+A33S+E34D+H35I+H156Y+A181T+N190F+A209V; A1*+N2*+L3V+M15T+R23K+S-29A+A30E+Y31H+A33S+E34D+H35I+H156Y+A181T+N190F+A209V+Q264S.

Claims Text - CLTX (3):

3. A variant according to claim 1 or 2, wherein the parent Termamyl-like .alpha.-amylase is selected from: the B. licheniformis .alpha.-amylase having the sequence shown in SEQ ID No. 2, the B. amyloliquefaciens .alpha.-amylase having the sequence shown in SEQ ID No. 4, the B. stearothermophilus .alpha.-amylase having the sequence shown in SEQ ID No. 6, the Bacillus strain NCIB 12512 .alpha.-amylase having the sequence shown in FIGS. 1 and 2, the Bacillus strain NCIB 12513 .alpha.-amylase having the sequence shown in FIG. 2, and the Bacillus sp. #707 .alpha.-amylase having the sequence shown in FIG. 2.

Claims Text - CLTX (23):

23. A method for generating a variant of a parent Termamyl-like .alpha.-amylase, which variant exhibits increased stability at low pH and at low calcium concentration relative to the parent, the method comprising: (a) subjecting a DNA sequence encoding the parent Termamyl-like .alpha.-amylase to random mutagenesis, (b) expressing the mutated DNA sequence obtained in step (a) in a host cell, and (c) screening for host cells expressing a mutated .alpha.-amylase which has increased stability at low pH and low calcium concentration relative to the parent .alpha.-amylase.

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PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030170769 A1

TITLE: Alpha-amylase mutants

PUBLICATION-DATE: September 11, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Svendsen, Allan	Birkeroed		DK	
Bisgard-Frantzen, Henrik	Lyngby		DK	
Borchert, Torben Vedel	Copenhagen N		DK	

APPL-NO: 10/ 184771

DATE FILED: June 28, 2002

RELATED-US-APPL-DATA:

child 10184771 A1 20020628

parent continuation-of 09636252 20000810 US GRANTED

parent-patent 6440716 US

child 09636252 20000810 US

parent continuation-of 09327563 19990608 US PENDING

child 09327563 19990608 US

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parent-patent 6022724 US

child 08683838 19960718 US

parent continuation-in-part-of 08600908 19960213 US GRANTED

parent-patent 5989169 US

child 08600908 19960213 US

parent a-371-of-international PCT/DK96/00057 19960205 WO UNKNOWN

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
DK	0128/95	1995DK-0128/95	February 3, 1995
DK	1192/95	1995DK-1192/95	October 23, 1995
DK	1256/95	1995DK-1256/95	November 10, 1995

US-CL-CURRENT: 435/22, 435/201 , 435/320.1 , 435/325 , 435/69.1

ABSTRACT:

The present invention relates to a method of constructing a variant of a parent Termamyl-like .alpha.-amylase, which variant has .alpha.-amylase activity and at least one altered property as compared to the parent .alpha.-amylase, comprises
i) analysing the structure of the parent Termamyl-like .alpha.-amylase to identify at least one amino acid residue or at least one structural part of the Termamyl-like .alpha.-amylase structure, which amino acid residue or structural part is believed to be of relevance for altering the property of the parent Termamyl-like .alpha.-amylase (as evaluated on the basis of structural or functional considerations),
ii) constructing a Termamyl-like .alpha.-amylase variant, which as compared to the parent Termamyl-like .alpha.-amylase, has been modified in the amino acid residue or structural part identified in i) so as to alter the property, and, optionally,
iii) testing the resulting Termamyl-like .alpha.-amylase variant with respect to the property in question.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation of U.S. application Ser. No. 09/636,252, filed on Aug. 10, 2000, which is a continuation of 09/327,563, filed on Jun. 8, 1999, which is continuation of Ser. No. 08/683,838, filed on Jul. 18,1996, now U.S. Pat. No. 6,022,724, which is a continuation-in-part of Ser. No. 08/600,908, filed on Feb. 13, 1996, now U.S. Pat. No. 5,989,169, which is a 371 of PCT/DK96/00057, filed on Feb. 5, 1996, and claims priority under 35 U.S.C. 119 of Danish applications 0128/95, filed on Feb. 3, 1995, 1192/95, filed on Oct. 23, 1995, and 1256/95, filed on Nov. 10, 1995, the contents of which are fully incorporated herein by reference.

---------- KWIC ---------

Pre-Grant Publication Document Identifier - DID (1):
US 20030170769 A1

Summary of Invention Paragraph - BSTX (361):
[0358] .alpha.-Amylase activity is detected by Cibacron Red labelled amylopectin, which is immobilized on agarose. For screening for variants with increased thermal and high-pH stability, the filter with bound .alpha.-amylase variants is incubated in a buffer at pH 10.5 and 60.degree. or 65.degree. C. for a specified time, rinsed briefly in deionized water and placed on the amylopectin-agarose matrix for activity detection. Residual activity is seen as lysis of Cibacron Red by amylopectin degradation. The conditions are chosen to be such that activity due to the .alpha.-amylase having the amino acid sequence shown in SEQ ID NO: 1 can barely be detected. Stabilized variants show, under the same conditions, increased colour intensity due to increased liberation of Cibacron Red.

Summary of Invention Paragraph - BSTX (368):
[0365] In the vector, the DNA sequence should be operably connected to a suitable promoter sequence. The promoter may be any DNA sequence which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. Examples of suitable promoters for directing the transcription of the DNA sequence encoding an .alpha.-amylase variant of the invention, especially in a bacterial host, are the promoter of the lac operon of E. coli, the Streptomyces coelicolor agarase gene dagA promoters, the promoters of the Bacillus licheniformis .alpha.-amylase gene (amyL), the promoters of the Bacillus

stearothermophilus maltogenic amylase gene (amyM), the promoters of the Bacillus amyloliquefaciens .alpha.-amylase (amyQ), the promoters of the Bacillus subtilis xylA and xylB genes etc. For transcription in a fungal host, examples of useful promoters are those derived from the gene encoding A. oryzae TAKA amylase, Rhizomucor miehei aspartic proteinase, A. niger neutral .alpha.-amylase, A. niger acid stable .alpha.-amylase, A. niger glucoamylase, Rhizomucor miehei lipase, A. oryzae alkaline protease, A. oryzae triose phosphate isomerase or A. nidulans acetamidase.

Detail Description Paragraph - DETX (47):

[0517] It is apparent from the above that the calcium-binding affinity of the variant in question binds calcium significantly more strongly than the parent, and thereby has a correspondingly lower calcium dependency than the parent.

PGPUB-DOCUMENT-NUMBER: 20030129718

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030129718 A1

TITLE: Amylase variants

PUBLICATION-DATE: July 10, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Andersen, Carsten	Vaerlose		DK	
Borchert, Torben Vedel	Birkerod		DK	
Nielsen, Bjarne Ronfeldt	Virum		DK	

APPL-NO: 09/ 925576

DATE FILED: August 9, 2001

RELATED-US-APPL-DATA:

child 09925576 A1 20010809

parent continuation-of PCT/DK01/00144 20010304 US UNKNOWN

non-provisional-of-provisional 60189857 20000315 US

non-provisional-of-provisional 60271382 20010226 US

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
DK	PA 2000 00376	2000DK-PA 2000 00376	March 8, 2000
DK	PA 2001 00303	2001DK-PA 2001 00303	February 23, 2001

US-CL-CURRENT: 435/183, 510/392

ABSTRACT:

The present invention relates to variants (mutants) of polypeptides, in particular Termamyl-like alpha-amylases, which variant has alpha-amylase activity and exhibits an alteration in at least one of the following properties relative to said parent alpha-amylase: substrate specificity, substrate binding, substrate cleavage pattern, thermal stability, pH/activity profile, pH/stability profile, stability towards oxidation, Ca.sup.2+ dependency, specific activity, and solubility, in particular under production conditions.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation of PCT/DK01/00144 filed Mar. 7, 2001 (the international application was published under PCT Article 21(2) in English) and claims, under 35 U.S.C. 119, priority or the benefit of Danish application nos. PA 2000 00376 and PA 2001 00303 filed Mar. 8, 2000 and Feb. 23, 2001, respectively, and U.S. provisional application Nos. 60/189,857, and 60/271,382 filed Mar. 15, 2000 and Feb. 26, 2001, the contents of which are fully incorporated herein by reference.

---------- KWIC --------

Pre-Grant Publication Document Identifier - DID
(1):
US 20030129718 A1

Detail Description Paragraph - DETX (293):
[0330] In the vector, the DNA sequence should be operably connected to a suitable promoter sequence. The promoter may be any DNA sequence, which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. Examples of suitable promoters for directing the transcription of the DNA sequence encoding an alpha-amylase variant of the invention, especially in a bacterial host, are the promoter of the lac operon of E. coli, the Streptomyces coelicolor agarase gene dagA promoters, the promoters of the Bacillus licheniformis alpha-amylase gene (amyL), the promoters of the Bacillus stearothermophilus maltogenic amylase gene (amyM), the promoters of the Bacillus amyloliquefaciens alpha-amylase (amyQ), the promoters of the Bacillus subtilis xylA and xylB genes etc. For transcription in a fungal host, examples of useful promoters are those derived from the gene encoding A. oryzae TAKA amylase, Rhizomucor miehei aspartic proteinase, A. niger neutral alpha-amylase, A. niger acid stable alpha-amylase, A. niger glucoamylase, Rhizomucor miehei lipase, A. oryzae alkaline protease, A. oryzae triose phosphate isomerase or A. nidulans acetamidase.

Detail Description Paragraph - DETX (374):
[0411] The assay can be used to screening of Termamyl-like .alpha.-amylase variants having an improved stability at high pH compared to the parent enzyme and Termamyl-like .alpha.-amylase variants having an improved stability at high pH and medium temperatures compared to the parent enzyme depending of the screening temperature setting.

Detail Description Paragraph - DETX (409):
[0446] 3. Decide on which kind of mutations should be carried out, e.g. with respect to the desired stability and/or performance of the variant to be constructed

Claims Text - CLTX (11):
11. The variant of any of claims 1-10, wherein the parent Termamyl-like alpha-amylase is derived from a strain of B. licheniformis, B. amyloliquefaciens, B. stearothermophilus, Bacillus sp. NCIB 12289, NCIB 12512, NCIB 12513 or DSM 9375, or DSMZ no. 12649, KSM AP1378, or KSM K36 or KSM K38.

US-PAT-NO: 6696402

DOCUMENT-IDENTIFIER: US 6696402 B2

TITLE: Laundry detergent compositions comprising zwitterionic polyamines

DATE-ISSUED: February 24, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Gosselink; Eugene Paul	Cincinnati	OH	N/A	N/A
Price; Kenneth Nathan	Cincinnati	OH	N/A	N/A

APPL-NO: 10/ 232357

DATE FILED: August 30, 2002

PARENT-CASE:

CROSS REFERENCE TO RELATED APPLICATIONS

This Application is a continuation that claims the benefit of U.S. application Ser. No. 10/129,618, filed on May 8, 2002, which in turn is a 371 of PCT International Application Serial No. PCT/US00/30645, filed Nov. 7, 2000, which claims the benefit of U.S. Provisional Application Serial No. 60/164,283 filed on Nov. 9, 1999, (now abandoned).

US-CL-CURRENT: 510/303, 510/309 , 510/310 , 510/311 , 510/312 , 510/336 , 510/340 , 510/341 , 510/350 , 510/351 , 510/356 , 510/360 , 510/499

ABSTRACT:

The present invention relates to laundry detergent compositions comprising: A) from about 0.01%, preferably from about 0.1%, more preferably from about 1%, most preferably from about 3% to about 50%, preferably to about 20%, more preferably to about 10%, most preferably to about 7% by weight, of a hydrophobically modified polyamine having the formula: ##STR1##

wherein R is C.sub.5 -C.sub.20 linear or branched alkylene, and mixtures thereof; R.sup.1 is an alkyleneoxy unit having the formula:

--(R.sup.2 O).sub.x --R.sup.3

wherein R.sup.2 is C.sub.2 -C.sub.4 linear or branched alkylene, and mixtures thereof; at least one R.sup.3 is an anionic unit, and the remaining R.sup.3 moieties are selected from the group consisting of hydrogen, C.sub.1 -C.sub.22 alkyl, C.sub.7 -C.sub.22 alkylenearyl, an anionic unit, and mixtures thereof; x is from about 15 to about 30; Q is a hydrophobic quaternizing unit selected from the group consisting of C.sub.8 -C.sub.30 linear or branched alkyl, C.sub.6 -C.sub.30 cycloalkyl, C.sub.7 -C.sub.30 substituted or unsubstituted alkylenearyl, and mixtures thereof; X is an anion present in sufficient amount to provide-electronic neutrality; n is from 0 to 4; B) from about 0.01% by weight, of a surfactant system comprising one or more surfactants selected from: i) from 0% to 100% by weight, of one or more anionic surfactants; ii) from 0% to 100% by weight, of one or more nonionic

surfactants; iii) optionally from 0.1% to about 80% by weight, of one or more cationic surfactants; iv) optionally from 0.1% to about 80% by weight, of one or more zwitterionic surfactants; v) optionally from 0.1% to about 80% by weight, of one or more ampholytic surfactants; or vi) mixtures thereof; C) the balance carriers and adjunct ingredients.

8 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

US Patent No. - PN (1):
6696402

Brief Summary Text - BSTX (129):
A preferred protease enzyme for use in the present invention is a variant of Protease A (BPN') which is a non-naturally occurring carbonyl hydrolase variant having a different proteolytic activity, stability, substrate specificity, pH profile and/or performance characteristic as compared to the precursor carbonyl hydrolase from which the amino acid sequence of the variant is derived. This variant of BPN' is disclosed in EP 130,756 A, Jan. 9, 1985. Specifically Protease A-BSV is BPN' wherein the Gly at position 166 is replaced with Asn, Ser, Lys, Arg, His, Gln, Ala, or Glu; the Gly at position 169 is replaced with Ser; the Met at position 222 is replaced with Gln, Phe, Cys, His, Asn, Glu, Ala or Thr; or alternatively the Gly at position 166 is replaced with Lys, and the Met at position 222 is replaced with Cys; or alternatively the Gly at position 169 is replaced with Ala and the Met at position 222 is replaced with Ala.

Brief Summary Text - BSTX (131):
A preferred protease enzyme for use in the present invention is Protease B. Protease B is a non-naturally occurring carbonyl hydrolase variant having a different proteolytic activity, stability, substrate specificity, pH profile and/or performance characteristic as compared to the precursor carbonyl hydrolase from which the amino acid sequence of the variant is derived. Protease B is a variant of BPN' in which tyrosine is replaced with leucine at position +217 and as further disclosed in EP 303,761 A, Apr. 28, 1987 and EP 130,756 A, Jan. 9, 1985.

Brief Summary Text - BSTX (154):
Amylases suitable herein include, for example, .alpha.-amylases described in GB 1,296,839 to Novo; RAPIDASE.RTM., International Bio-Synthetics, Inc. and TERMAMYL.RTM., Novo. FUNGAMYL.RTM. from Novo is especially useful. Engineering of enzymes for improved stability, e.g., oxidative stability, is known. See, for example J. Biological Chem., Vol. 260, No. 11, June 1985, pp 6518-6521 and WO 9402597 to Novo, Feb. 3, 1994, and WO 9509909 A to Novo. Certain preferred embodiments of the present compositions can make use of amylases having improved stability in detergents, especially improved oxidative stability as measured against a reference-point of TERMAMYL.RTM. in commercial use in 1993. These preferred amylases herein share the characteristic of being "stability-enhanced" amylases, characterized, at a minimum, by a measurable improvement in one or more of: oxidative stability, e.g., to hydrogen peroxide/tetraacetylethylenediamine in buffered solution at pH 9-10; thermal stability, e.g., at common wash temperatures such as about 60.degree. C.; or alkaline stability, e.g., at a pH from about 8 to about 11, measured versus the above-identified reference-point amylase. Stability can be measured using any of the art-disclosed technical tests. See, for example, references disclosed in WO 9402597. Stability-enhanced amylases can be obtained from Novo or from

Genencor International. One class of highly preferred amylases herein have the commonality of being derived using site-directed mutagenesis from one or more of the Baccillus amylases, especially the Bacillus alpha.-amylases, regardless of whether one, two or multiple amylase strains are the immediate precursors. Oxidative stability-enhanced amylases vs. the above-identified reference amylase are preferred for use, especially in bleaching, more preferably oxygen bleaching, as distinct from chlorine bleaching, detergent compositions herein. Such preferred amylases include (a) an amylase according to the hereinbefore incorporated WO 9402597, Novo, Feb. 3, 1994, as further illustrated by a mutant in which substitution is made, using alanine or threonine, preferably threonine, of the methionine residue located in position 197 of the B.licheniformis alpha-amylase, known as TERMAMYL.RTM., or the homologous position variation of a similar parent amylase, such as B. amyloliquefaciens, B. subtilis, or B. stearothermophilus; (b) stability-enhanced amylases as described by Genencor International in a paper entitled "Oxidatively Resistant alpha-Amylases" presented at the 207th American Chemical Society National Meeting, Mar. 13-17 1994, by C. Mitchinson. Therein it was noted that bleaches in automatic dishwashing detergents inactivate alpha-amylases but that improved oxidative stability amylases have been made by Genencor from B.licheniformis NCIB8061. Methionine (Met) was identified as the most likely residue to be modified. Met was substituted, one at a time, in positions 8, 15, 197, 256, 304, 366 and 438 leading to specific mutants, particularly important being M197L and M197T with the M197T variant being the most stable expressed variant. Stability was measured in CASCADE.RTM. and SUNLIGHT.RTM.; (c) particularly preferred amylases herein include amylase variants having additional modification in the immediate parent as described in WO 9510603 A and are available from the assignee, Novo, as DURAMYL.RTM.. Other particularly preferred oxidative stability enhanced amylase include those described in WO 9418314 to Genencor International and WO 9402597 to Novo. Any other oxidative stability-enhanced amylase can be used, for example as derived by site-directed mutagenesis from known chimeric, hybrid or simple mutant parent forms of available amylases. Other preferred enzyme modifications are accessible. See WO 9509909 A to Novo.

US-PAT-NO: 6673589

DOCUMENT-IDENTIFIER: US 6673589 B2

TITLE: .alpha.-amylase mutants

DATE-ISSUED: January 6, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Borchert; Torben Vedel	Copenhagen .O slashed.	N/A	N/A	DK
Svendsen; Allan	Birker.o slashed.d	N/A	N/A	DK
Andersen; Carsten	Vaerloese	N/A	N/A	DK
Nielsen; Bjarne	Virum	N/A	N/A	DK
Nissen; Torben Lauesgaard	Frederiksberg C	N/A	N/A	DK
Kj.ae butted.rulff; S.o slashed.ren	Vanl.o slashed.se	N/A	N/A	DK

APPL-NO: 09/ 769864

DATE FILED: January 25, 2001

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a divisional of U.S. Ser. No. 09/183,412 filed on Oct. 30, 1998, now U.S. Pat. No. 6,204,232 and claims priority under 35 U.S.C. 119 of Danish application no. 1240/97 filed on Oct. 30, 1997, Danish application no. PA 1998 00936 filed on Jul. 14, 1998, U.S. provisional application No. 60/064,662 filed on Nov. 6, 1997 and U.S. provisional application No. 60/093,234 filed on Jul. 17, 1998, the contents of which are fully incorporated herein by reference.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DK	1240/97	October 30, 1997
DK	PA 1998 00936	July 14, 1998

US-CL-CURRENT: 435/202, 510/226 , 510/236 , 510/320 , 510/396

ABSTRACT:

The invention relates to a variant of a parent Termamyl-like .sub..alpha. -amylase, which exhibits an alteration in at least one of the following properties relative to said parent .sub..alpha. -amylase: i) improved pH stability at a pH from 8 to 10.5; and/or ii) improved Ca.sup.2+ stability at pH 8 to 10.5, and/or iii) increased specific activity at temperatures from 10 to 60.degree. C.

34 Claims, 7 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 7

---------- KWIC ---------

US Patent No. - PN (1):
6673589

Brief Summary Text - BSTX (14):
Alterations in properties which may be achieved in variants(mutants) of the invention are alterations in: the stability of the Termamyl-like .sub..alpha. -amylase at a pH from 8 to 10.5, and/or the Ca.sup.2+ stability at pH 8 to 10.5, and/or the specific activity at temperatures from 10 to 60.degree. C., preferably 20-50.degree. C., especially 30-40.degree. C.

Detailed Description Text - DETX (49):
Preferred high pH stability variants include one or more of the following substitutions in the SP722 .sub..alpha. -amylase (having the amino acid sequence shown in SEQ ID NO: 2):

Detailed Description Text - DETX (53):
.sub..alpha. -amylase variants with improved stability at high pH can be constructed by making substitutions in the regions found using the molecular dynamics simulation mentioned in Example 2. The simulation depicts the region(s) that has a higher flexibility or mobility at high pH (i.e., pH 8-10.5) when compared to medium pH.

Detailed Description Text - DETX (210):
The assay can be used to screening of Termamyl-like .sub..alpha. -amylase variants having an improved stability at high pH compared to the parent enzyme and Termamyl-like .sub..alpha. -amylase variants having an improved stability at high pH and medium temperatures compared to the parent enzyme depending of the screening temperature setting

Detailed Description Text - DETX (241):
3. Decide on which kind of mutations should be carried out, e.g. with respect to the desired stability and/or performance of the variant to be constructed

Detailed Description Text - DETX (265):
Method of extracting important regions for identifying .sub..alpha. -amylase variants with improved pH stability and altered temperature activity

Detailed Description Text - DETX (268):
1. The approach used for extracting important regions for identifying .sub..alpha. -amylase variants with high pH stability:

Detailed Description Text - DETX (269):
The important regions for constructing variants with improved pH stability are the regions which at the extreme pH display the highest mobility, i.e., regions having the highest isotropic fluctuations.

Detailed Description Text - DETX (276):
Construction, by localized random, doped mutagenesis, of Termamyl-like .sub..alpha. -amylase variants having an improved Ca2+ stability at medium temperatures compared to the parent enzyme

Detailed Description Text - DETX (420):
Determination of pH stability at alkaline pH of variants of the parent .sub..alpha. -Amylase having the amino acid sequence shown in SEQ ID NO:2.

Detailed Description Text - DETX (428):

Determination of calcium stability at alkaline pH of variants of the parent .sub..alpha. -Amylase having the amino acid sequence shown in SEQ ID NO: 1, SEQ ID NO: 2 and SEQ ID NO: 4.

Detailed Description Text - DETX (429):
A: Calcium stability of variants of the sequence in SEQ ID NO: 1

Detailed Description Text - DETX (433):
B: Calcium stability of variants of the sequence in SEQ ID NO: 2

Detailed Description Text - DETX (440):
C: Calcium stability of variants of the sequence in SEQ ID NO: 4

Claims Text - CLTX (3):
3. The variant according to claim 1, wherein said variant exhibits improved stability at pH 8 to 10.5 as compared to said parent .alpha.-amylase.

Claims Text - CLTX (4):
4. The variant according to claim 1, wherein said variant exhibits improved Ca.sup.2+ stability at pH 8 to 10.5 as compared to said parent .alpha.-amylase.

Claims Text - CLTX (5):
5. The variant according to claim 1, wherein the parent Termamyl-like .alpha.-amylase is selected from the group consisting of: (i) Bacillus strain NCIB 12512 .alpha.-amylase having the sequence shown in SEQ ID NO: 1; (ii) B. amyloliquefaciens .alpha.-amylase having the sequence shown in SEQ ID NO: 5; and (iii) B. licheniformis .alpha.-amylase having the sequence shown in SEQ ID NO: 4.

US-PAT-NO: 6667288

DOCUMENT-IDENTIFIER: US 6667288 B2

TITLE: Bleach compositions

DATE-ISSUED: December 23, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Burns; Michael Eugene	Hamilton	OH	N/A	N/A
Graydon; Andrew Russell	Tyne & Wear	N/A	N/A	GB
Labeque; Regine	Brussels	N/A	N/A	BE
Perkins; Christopher Mark	Cincinnati	OH	N/A	N/A
Sadlowski; Eugene Steven	Cincinnati	OH	N/A	N/A
Williams; Barbara Kay	Cincinnati	OH	N/A	N/A

APPL-NO: 10/ 142041

DATE FILED: May 9, 2002

PARENT-CASE:

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation under 35 USC .sctn.120 to U.S. application Ser. No. 09/831,607, filed May 10, 2001, now abandoned, which is an entry into the U.S. National Stage under 35 U.S.C. .sctn.371 of PCT International Application Serial No. PCT/US99/26543, filed Nov. 9, 1999, which claims priority under PCT Article 8 and 35 U.S.C. .sctn.119(e) to U.S. Provisional Application Ser. No. 60/108,292 filed Nov. 13, 1998, (now abandoned.

US-CL-CURRENT: 510/311, 252/186.33 , 510/220 , 510/221 , 510/224 , 510/303 , 510/372 , 510/376 , 8/111 , 8/137

ABSTRACT:

The present invention relates to bleaching, pre-soak, pre-treatment, and laundry detergent compositions comprising: A) a catalytically effective amount of a transition-metal bleach catalyst which is a complex of a transition-metal and a cross-bridged macropolycyclic ligand, for example. 5,12-dimethyl-1,5,8,12-tetraazabicyclo[6.6.2] hexadecane manganese (II) chloride, having the formula: ##STR1## B) the balance carriers and other adjunct ingredients;

provided said composition is substantially free of any organic or inorganic peroxygen compounds.

25 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

US Patent No. - PN (1):

Brief Summary Text - BSTX (122):

Amylases (.alpha. and/or .beta.) can be included for removal of carbohydrate-based stains. WO94/02597 describes cleaning compositions which incorporate mutant amylases. See also WO95/10603. Other amylases known for use in cleaning compositions include both .alpha.- and .beta.-amylases. .alpha.-Amylases are known in the art and include those disclosed in U.S. Pat. No. 5,003,257; EP 252,666; WO/91/00353; FR 2,676,456; EP 285,123; EP 525,610; EP 368,341; and British Patent specification no. 1,296,839 (Novo). Other suitable amylases are stability-enhanced amylases described in WO94/18314 and WO96/05295, Genencor, and amylase variants having additional modification in the immediate parent available from Novo Nordisk A/S, disclosed in WO 95/10603. Also suitable are amylases described in EP 277 216.

Brief Summary Text - BSTX (125):

The above-mentioned enzymes may be of any suitable origin, such as vegetable, animal, bacterial, fungal and yeast origin. Origin can further be mesophilic or extremophilic (psychrophilic, psychrotrophic, thermophilic, barophilic, alkalophilic, acidophilic, halophilic, etc.). Purified or non-purified forms of these enzymes may be used Nowadays, it is common practice to modify wild-type enzymes via protein/genetic engineering techniques in order to optimize their performance efficiency in the laundry detergent and/or fabric care compositions of the invention. For example, the variants may be designed such that the compatibility of the enzyme to commonly encountered ingredients of such compositions is increased. Alternatively, the variant may be designed such that the optimal pH, bleach or chelant stability, catalytic activity and the like, of the enzyme variant is tailored to suit the particular cleaning application.

Brief Summary Text - BSTX (132):

Suitable .alpha.-amylase variants for use in the present invention include, but are not limited to the following .alpha.-amylases: (i) .alpha.-amylase characterized by having a specific activity at least 25% higher than the specific activity of Termamyl.RTM. at a temperature range of 25.degree. C. to 55.degree. C. and at a pH value in the range of 8 to 10, measured by Phadebas.RTM. .alpha.-amylase activity assay and/or; (ii) .alpha.-amylase according to (i) comprising the amino acid sequence shown in SEQ ID No. 1 or an .alpha.-amylase being at least 80% homologous with the amino acid sequence shown in SEQ ID No. 1 and/or; (iii) .alpha.-amylase according to (i) comprising the amino acid sequence shown in SEQ ID No. 2 or an .alpha.-amylase being at least 80% homologous with the amino acid sequence shown in SEQ ID No. 2 and/or; (iv) .alpha.-amylase according to (i) comprising the following amino acid sequence N-terminal:
His-His-Asn-Gly-Thr-Asn-Gly-Thr-Met-Met-Gln-Tyr-Phe-Glu-Trp-Tyr-Leu-Pro-As
n-Asp (SEQ ID No. 3) or an .alpha.-amylase being at least 80% homologous with the amino acid sequence shown (SEQ ID No. 3) in the N-terminal and/or; (v) .alpha.-amylase according to (i-iv) wherein the .alpha.-amylase is obtainable from an alkalophilic Bacillus species and/or; (vi) .alpha.-amylase according to (v) wherein the amylase is obtainable from any of the strains NCIB 12289, NCIB 12512, NCIB 12513 and DSM 935 and/or; (vii) .alpha.-amylase showing positive immunological cross-reactivity with antibodies raised against an .alpha.-amylase having an amino acid sequence corresponding respectively to SEQ ID No. 1, ID No. 2, or ID No. 3 and/or, (viii) variant of a parent .alpha.-amylase, wherein the parent .alpha.-amylase (1) has one of the amino acid sequences shown in SEQ ID No. 1, ID No. 2, or ID No. 4, respectively, or (2) displays at least 80% homology with one or more of said amino acid sequences, and/or displays immunological cross-reactivity with an antibody raised against an .alpha.-amylase having one of said amino acid sequences,

and/or is encoded by a DNA sequence which hybridizes with the same probe as a DNA sequence encoding an .alpha.-amylase having one of said amino acid sequences, in which variants: (A) at least one amino acid residue of said parent .alpha.-amylase has been deleted; and/or (B) at least one amino acid residue of said parent .alpha.-amylase has been replaced by a different amino acid residue; and/or (C) at least one amino acid residue has been inserted relative to said parent .alpha.-amylase; said variant having an .alpha.-amylase activity and exhibiting at least one of the following properties relative to said parent .alpha.-amylase: increased thermostability; increased stability towards oxidation; reduced Ca ion dependency; increased stability and/or .alpha.-amylolytic activity at neutral to relatively high pH values; increased .alpha.-amylolytic activity at relatively high temperature; and increase or decrease of the isoelectric point (pI) so as to better match the pI value for .alpha.-amylase variant to the pH of the medium.

US-PAT-NO: 6660711

DOCUMENT-IDENTIFIER: US 6660711 B1

TITLE: Laundry detergent compositions comprising zwitterionic polyamines and mid-chain branched surfactants

DATE-ISSUED: December 9, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Price; Kenneth Nathan	Cincinnati	OH	N/A	N/A
Gosselink; Eugene Paul	Cincinnati	OH	N/A	N/A

APPL-NO: 09/ 980799

DATE FILED: December 3, 2001

PARENT-CASE:

This application claims priority under 35 USC 119(e) to U.S. Provision appl. No.(s) 60/160,431, filed Oct. 19, 1999, Ser. No. 60/160,324, filed Oct. 19, 1999, Ser. No. 60/160,272, filed Oct. 19, 1999, Ser. No. 60/160,289, filed Oct. 19, 1999, Ser. No. 60/144,321, filed Jul. 16, 1999, Ser. No. 60/144,110, filed Jul. 16, 1999, Ser. No. 60/144,113, filed Jul. 16, 1999, and Ser. No. 60/144,111, filed Jul. 16, 1999.

PCT-DATA:
APPL-NO: PCT/US00/19084
DATE-FILED: July 13, 2000
PUB-NO: WO01/05923
PUB-DATE: Jan 25, 2001
371-DATE:
102(E)-DATE:

US-CL-CURRENT: 510/499, 510/303 , 510/309 , 510/310 , 510/311 , 510/312 , 510/321 , 510/337 , 510/338 , 510/393 , 510/504

ABSTRACT:

The present invention relates to laundry detergent compositions which provide enhanced hydrophilic soil cleaning benefits, said compositions comprising from about 0.01% by weight of a zwitterionic polyamine, b) from about 0.01% by weight of a surfactant system; c) and the balance, adjunct ingredients.

11 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

US Patent No. - PN (1):
6660711

Detailed Description Text - DETX (102):

A preferred protease enzyme for use in the present invention is a variant of Protease A (BPN') which is a non-naturally occurring carbonyl hydrolase variant having a different proteolytic activity, stability, substrate specificity, pH profile and/or performance characteristic as compared to the precursor carbonyl hydrolase from which the amino acid sequence of the variant is derived. This variant of BPN' is disclosed in EP 130,756 A, Jan. 9, 1985. Specifically Protease A-BSV is BPN' wherein the Gly at position 166 is replaced with Asn, Ser, Lys, Arg, His, Gln, Ala, or Glu; the Gly at position 169 is replaced with Ser; the Met at position 222 is replaced with Gln, Phe, Cys, His, Asn, Glu, Ala or Thr; or alternatively the Gly at position 166 is replaced with Lys, and the Met at position 222 is replaced with Cys; or alternatively the Gly at position 169 is replaced with Ala and the Met at position 222 is replaced with Ala.

Detailed Description Text - DETX (104):

A preferred protease enzyme for use in the present invention is Protease B. Protease B is a non-naturally occurring carbonyl hydrolase variant having a different proteolytic activity, stability, substrate specificity, pH profile and/or performance characteristic as compared to the precursor carbonyl hydrolase from which the amino acid sequence of the variant is derived. Protease B is a variant of BPN' in which tyrosine is replaced with leucine at position +217 and as further disclosed in EP 303,761 A, Apr. 28, 1987 and EP 130,756 A, Jan. 9, 1985.

Detailed Description Text - DETX (129):

Amylases suitable herein include, for example, .alpha.-amylases described in GB 1,296,839 to Novo; RAPIDASE.RTM., International Bio-Synthetics, Inc. and TERMAMYL.RTM., Novo. FUTNGAMYL.RTM. from Novo is especially useful. Engineering of enzymes for improved stability, e.g., oxidative stability, is known. See, for example J. Biological Chem., Vol. 260, No. 11, June 1985, pp 6518-6521. Certain preferred embodiments of the present compositions can make use of amylases having improved stability in detergents, especially improved oxidative stability as measured against a reference-point of TERMAMYL.RTM. in commercial use in 1993. These preferred amylases herein share the characteristic of being "stability-enhanced" amylases, characterized, at a minimum, by a measurable improvement in one or more of: oxidative stability, e.g., to hydrogen peroxide/tetraacetylethylenediamine in buffered solution at pH 9-10; thermal stability, e.g., at common wash temperatures such as about 60.degree. C.; or alkaline stability, e.g., at a pH from about 8 to about 11, measured versus the above-identified reference-point amylase. Stability can be measured using any of the art-disclosed technical tests. See, for example, references disclosed in WO 9402597. Stability-enhanced amylases can be obtained from Novo or from Genencor International. One class of highly preferred amylases herein have the commonality of being derived using site-directed mutagenesis from one or more of the Baccillus amylases, especially the Bacillus .alpha.-amylases, regardless of whether one, two or multiple amylase strains are the immediate precursors. Oxidative stability-enhanced amylases vs. the above-identified reference amylase are preferred for use, especially in bleaching, more preferably oxygen bleaching, as distinct from chlorine bleaching, detergent compositions herein. Such preferred amylases include (a) an amylase according to the hereinbefore incorporated WO 9402597, Novo, Feb. 3, 1994, as further illustrated by a mutant in which substitution is made, using arginine or threonine, preferably threonine, of the methionine residue located in position 197 of the B.licheniformis alpha-amylase, known as TERMAMYL.RTM., or the homologous position variation of a similar parent amylase, such as B. amyloliquefaciens, B. subtilis, or B. stearothennophilus; (b) stability-enhanced amylases as described by Genencor International in a paper entitled "Oxidatively Resistant alpha-Amylases" presented at the 207th American Chemical Society National Meeting, Mar. 13-17, 1994, by C. Mitchinson. Therein it was noted that

bleaches in automatic dishwashing detergents inactivate alpha-amylases but that improved oxidative stability amylases have been made by Genencor from B. licheniformis NCIB8061. Methionine (Met) was identified as the most likely residue to be modified. Met was substituted, one at a time, in positions 8, 15, 197, 256, 304, 366 and 438 leading to specific mutants, particularly important being M197L and M197T with the M197T variant being the most stable expressed variant. Stability was measured in CASCADE.RTM. and SUNLIGHT.RTM.; (c) particularly preferred amylases herein include amylase variants having additional modification in the immediate parent as described in WO 9510603 A and are available from the assignee, Novo, as DURAMYL.RTM.D. Other particularly preferred oxidative stability enhanced amylase include those described in WO 9418314 to Genencor International and WO 9402597 to Novo. Any other oxidative stability-enhanced amylase can be used, for example as derived by site-directed mutagenesis from known chimeric, hybrid or simple mutant parent forms of available amylases. Other preferred enzyme modifications are accessible. See WO 9509909 A to Novo.

US-PAT-NO: 6653270

DOCUMENT-IDENTIFIER: US 6653270 B2

TITLE: Stabilized bleach compositions

DATE-ISSUED: November 25, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
LaBeque; Regine	Brussels	N/A	N/A	BE

APPL-NO: 10/ 142070

DATE FILED: May 9, 2002

PARENT-CASE:

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation under 35 USC .sctn.120 to U.S. application Ser. No. 09/914,528, filed Aug. 29, 2001, now abandoned which is an entry into the U.S. National Stage under 35 U.S.C. .sctn.371 of PCT International Application Ser. No. PCT/US00/05291, filed Feb. 29, 2000 which claims priority under PCT Article 8 and 35 U.S.C. .sctn. 119(e) to U.S. Provisional Application Ser. No. 60/122,492 filed Mar. 2, 1999, (now abandoned).

US-CL-CURRENT: 510/311, 252/186.33 , 510/220 , 510/224 , 510/302 , 510/303 , 510/312 , 510/372 , 510/376 , 8/111 , 8/137

ABSTRACT:

The present invention relates to bleaching, pre-soak, pre-treatment, and laundry detergent compositions comprising: A) a catalytically effective amount of a transition-metal bleach catalyst which is a complex of a transition-metal and a cross-bridged macropolycyclic ligand, for example, 5,12-dimethyl-1,5,8,12-tetraazabicyclo[6.6.2] hexadecane manganese (II) chloride, having the formula: ##STR1## B) an effective amount of a stabilizing agent, said agent selected from i) one or more anti-oxidants; ii) one or more reducing agents; iii) and mixtures thereof; and C) the balance carriers and other adjunct ingredients;

provided said composition is substantially free of any organic or inorganic peroxygen compounds.

35 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

US Patent No. - PN (1):
6653270

Brief Summary Text - BSTX (107):

Amylases (.alpha. and/or .beta.) can be included for removal of carbohydrate-based stains. WO94/02597 describes cleaning compositions which incorporate mutant amylases. See also WO95/10603. Other amylases known for use in cleaning compositions include both .alpha.- and .beta.-amylases. .alpha.-Amylases are known in the art and include those disclosed in US Pat. no. 5,003,257; EP 252,666; WO/91/00353; FR 2,676,456; EP 285,123; EP 525,610; EP 368,341; and British Patent specification no. 1,296,839 (Novo). Other suitable amylases are stability-enhanced amylases described in WO94/18314 and WO96/05295, Genencor, and amylase variants having additional modification in the immediate parent available from Novo Nordisk A/S, disclosed in WO 95/10603. Also suitable are amylases described in EP 277 216.

Brief Summary Text - BSTX (110):

The above-mentioned enzymes may be of any suitable origin, such as vegetable, animal, bacterial, fungal and yeast origin. Origin can further be mesophilic or extremophilic (psychrophilic, psychrotrophic, thermophilic, barophilic, alkalophilic, acidophilic, halophilic, etc.). Purified or non-purified forms of these enzymes may be used. Nowadays, it is common practice to modify wild-type enzymes via protein/genetic engineering techniques in order to optimize their performance efficiency in the laundry detergent and/or fabric care compositions of the invention. For example, the variants may be designed such that the compatibility of the enzyme to commonly encountered ingredients of such compositions is increased. Alternatively, the variant may be designed such that the optimal pH, bleach or chelant stability, catalytic activity and the like, of the enzyme variant is tailored to suit the particular cleaning application.

Brief Summary Text - BSTX (117):

Suitable .alpha.-amylase variants for use in the present invention include, but are not limited to the following .alpha.-amylases: (i) .alpha.-amylase characterized by having a specific activity at least 25% higher than the specific activity of Termamyl.RTM. at a temperature range of 25.degree. C. to 55.degree. C. and at a pH value in the range of 8 to 10, measured by Phadebas.RTM. .alpha.-amylase activity assay and/or; (ii) .alpha.-amylase according to (i) comprising the amino acid sequence shown in SEQ ID No. 1 or an .alpha.-amylase being at least 80% homologous with the amino acid sequence shown in SEQ ID No. 1 and/or; (iii) .alpha.-amylase according to (i) comprising the amino acid sequence shown in SEQ ID No. 2 or an .alpha.-amylase being at least 80% homologous with the amino acid sequence shown in SEQ ID No. 2 and/or; (iv) .alpha.-amylase according to (i) comprising the following amino acid sequence N-terminal:
His-His-Asn-Gly-Thr-Asn-Gly-Thr-Met-Met-Gln-Tyr-Phe-Glu-Trp-Tyr-Leu-Pro-As n-Asp (SEQ ID No. 3) or an .alpha.-amylase being at least 80% homologous with the amino acid sequence shown (SEQ ID No. 3) in the N-terminal and/or; (v) .alpha.-amylase according to (i-iv) wherein the .alpha.-amylase is obtainable from an alkalophilic Bacillus species and/or; (vi) .alpha.-amylase according to (v) wherein the amylase is obtainable from any of the strains NCIB 12289, NCIB 12512, NCIB 12513 and DSM 935 and/or; (vii) .alpha.-amylase showing positive immunological cross-reactivity with antibodies raised against an .alpha.-amylase having an amino acid sequence corresponding respectively to SEQ ID No. 1, ID No. 2, or ID No. 3 and/or; (viii) variant of a parent .alpha.-amylase, wherein the parent .alpha.-amylase (1) has one of the amino acid sequences shown in SEQ ID No. 1, ID No. 2, or ID No. 4, respectively, or (2) displays at least 80% homology with one or more of said amino acid sequences, and/or displays immunological cross-reactivity with an antibody raised against an .alpha.-amylase having one of said amino acid sequences, and/or is encoded by a DNA sequence which hybridizes with the same probe as a DNA sequence encoding an .alpha.-amylase having one of said amino acid sequences, in which variants: (A) at least one amino acid residue of said

parent .alpha.-amylase has been deleted; and/or (B) at least one amino acid residue of said parent a:-amylase has been replaced by a different amino acid residue; and/or (C) at least one amino acid residue has been inserted relative to said parent .alpha.-amylase; said variant having an .alpha.-amylase activity and exhibiting at least one of the following properties relative to said parent .alpha.-amylase: increased thermostability; increased stability towards oxidation; reduced Ca ion dependency; increased stability and/or .alpha.-amylolytic activity at neutral to relatively high pH values; increased .alpha.-amylolytic activity at relatively high temperature; and increase or decrease of the isoelectric point (pI) so as to better match the pI value for .alpha.-amylase variant to the pH of the medium.

US-PAT-NO: 6642044

DOCUMENT-IDENTIFIER: US 6642044 B2

TITLE: .alpha.-amylase mutants

DATE-ISSUED: November 4, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Svendsen; Allan	Birker.o slashed.d	N/A	N/A	DK
Borchert; Torben Vedel	Jyllinge	N/A	N/A	DK
Bisgard-Frantzen; Henrik	Bagsvaerd	N/A	N/A	DK

APPL-NO: 10/ 186042

DATE FILED: June 28, 2002

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a division of 09/672,459, filed on Sep. 28, 2000 (now U.S. Pat. No. 6,436,888), which is a continuation of 09/182,859, filed on Oct. 29, 1998 (now U.S. Pat. No. 6,143,708), which is a continuation of PCT/DK97/00197 filed Apr. 30, 1997 which claims priority under 35 U.S.C. 119 of Danish applications 0515/96 filed Apr. 30, 1996, 0712/96 filed Jun. 28, 1996, 0775/96 filed Jul. 11, 1996, and 1263/96 filed Nov. 8, 1996, the contents of which are fully incorporated herein by reference.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DK	0515/96	April 30, 1996
DK	0712/96	June 28, 1996
DK	0775/96	July 11, 1996
DK	1263/96	November 8, 1996

US-CL-CURRENT: 435/252.3, 435/202 , 435/320.1 , 510/226 , 536/23.2 , 536/23.7

ABSTRACT:

The invention relates to a variant of a parent Termamyl-like a-amylase, which variant has a-amylase activity and exhibits an alteration in at least one of the following properties relative to said parent a-amylase: substrate specificity, substrate binding, substrate cleavage pattern, thermal stability, pH/activity profile, pH/stability profile, stability towards oxidation, Ca.sup.2+ dependency and specific activity.

6 Claims, 9 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 9

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US Patent No. - PN (1):
6642044

Brief Summary Text - BSTX (5):
Among more recent disclosures relating to .alpha.-amylases, WO 96/23874 provides three-dimensional, X-ray crystal structural data for a Termamyl-like .alpha.-amylase which consists of the 300 N-terminal amino acid residues of the B. amyloliquefaciens .alpha.-amylase comprising the amino acid sequence shown in SEQ ID No. 4 herein and amino acids 301-483 of the C-terminal end of the B. licheniformis .alpha.-amylase comprising the amino acid sequence shown in SEQ ID No. 2 herein (the latter being available commercially under the tradename Termamyl.TM.), and which is thus closely related to the industrially important Bacillus .alpha.-amylases (which in the present context are embraced within the meaning of the term "Termamyl-like .alpha.-amylases", and which include, inter alia, the B. licheniformis, B. amyloliquefaciens and B. stearothermophilus .alpha.-amylases). WO 96/23874 further describes methodology for designing, on the basis of an analysis of the structure of a parent Termamyl-like .alpha.-amylase, variants of the parent Termamyl-like .alpha.-amylase which exhibit altered properties relative to the parent.

Brief Summary Text - BSTX (97):
Furthermore, it is preferred that the mutagenesis is carried out by use of doped or spiked oligonucleotides. The doping is preferably done so as to introduce amino acids contributing to improved stability at low pH and reduced calcium dependency at low pH of the resulting .alpha.-amylase variant. Furthermore, when selecting the doping scheme, the possibility of introducing Asn and Gln residues should generally be avoided, since Asn and Gln residues in general are associated with instability at low pH. Preferably, when a Pro residue can be inserted with potential benefits (e.g. as assessed from protein-structural considerations), the doping scheme is prepared to include a preference for introduction of a Pro residue.

Brief Summary Text - BSTX (99):
In relation to the above, a further aspect of the present invention relates to a method for generating a variant of a parent Termamyl-like .alpha.-amylase, which variant exhibits increased stability at low pH and at low calcium concentration relative to the parent, the method comprising: (a) subjecting a DNA sequence encoding the parent Termamyl-like .alpha.-amylase to random mutagenesis, (b) expressing the mutated DNA sequence obtained in step (a) in a host cell, and (c) screening for host cells expressing a mutated .alpha.-amylase which has increased stability at low pH and low calcium concentration relative to the parent .alpha.-amylase.

Brief Summary Text - BSTX (123):
.alpha.-Amylase activity is detected by Cibacron Red labelled amylopectin, which is immobilized on agarose. For screening for variants with increased thermal and high-pH stability, the filter with bound .alpha.-amylase variants is incubated in a buffer at pH 10.5 and 60.degree. or 65.degree. C. for a specified time, rinsed briefly in deionized water and placed on the amylopectin-agarose matrix for activity detection. Residual activity is seen as lysis of Cibacron Red by amylopectin degradation. The conditions are chosen to be such that activity due to the .alpha.-amylase having the amino acid sequence shown in SEQ ID No. 2 can barely be detected. Stabilized variants show, under the same conditions, increased colour intensity due to increased liberation of Cibacron Red.

Brief Summary Text - BSTX (130):
In the vector, the DNA sequence should be operably connected to a suitable

promoter sequence. The promoter may be any DNA sequence which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. Examples of suitable promoters for directing the transcription of the DNA sequence encoding an .alpha.-amylase variant of the invention, especially in a bacterial host, are the promoter of the lac operon of E.coli, the Streptomyces coelicolor agarase gene dagA promoters, the promoters of the Bacillus licheniformis .alpha.-amylase gene (amyL), the promoters of the Bacillus stearothermophilus maltogenic amylase gene (amyM), the promoters of the Bacillus amyloliquefaciens .alpha.-amylase (amyQ), the promoters of the Bacillus subtilis xylA and xylB genes etc. For transcription in a fungal host, examples of useful promoters are those derived from the gene encoding A. oryzae TAKA amylase, Rhizomucor miehei aspartic proteinase, A. niger neutral .alpha.-amylase, A. niger acid stable .alpha.-amylase, A. niger glucoamylase, Rhizomucor miehei lipase, A. oryzae alkaline protease, A. oryzae triose phosphate isomerase or A. nidulans acetamidase.

Detailed Description Text - DETX (13):
The random mutagenesis may be carried out by the following steps: 1. Select regions of interest for modification in the parent enzyme 2. Decide on mutation sites and nonmutated sites in the selected region 3. Decide on which kind of mutations should be carried out, e.g. with respect to the desired stability and/or performance of the variant to be constructed 4. Select structurally reasonable mutations. 5. Adjust the residues selected by step 3 with regard to step 4. 6. Analyze by use of a suitable dope algoritm the nucleotide distribution. 7. If necessary, adjust the wanted residues to genetic code realism (e.g. taking into account constraints resulting from the genetic code (e.g. in order to avoid introduction of stop codons))(the skilled person will be aware that some codon combinations cannot be used in practice and will need to be adapted) 8. Make primers 9. Perform random mutagenesis by use of the primers 10. Select resulting .alpha.-amylase variants by screening for the desired improved properties.

Detailed Description Text - DETX (56):
Construction, by Localized Random, Doped Mutagenesis, of Termamyl-like .alpha.-Amylase Variants Having an Improved Stability at Low pH and a Reduced Dependency on Calcium Ions for Stability Compared to the Parent Enzyme

Detailed Description Text - DETX (58):
has a very satisfactory stability at low pH and low calcium concentrations. In an attempt to further improve the stability at low pH and low calcium concentration of said .alpha.-amylase variant random mutagenesis in preselected regions wase performed.

Detailed Description Text - DETX (74):
The mutations indicated in bold were introduced by the random mutagenesis method. The stability data for these variants appear from Table 11 in Example 3.

Detailed Description Text - DETX (90):
This example summarises the stability results of variants characterised by a fluorimetric assay at 70.degree. C. under two different conditions, (1) pH 4.5 and 1 mM CaCl.sub.2 and (2) pH 6.2 and 10 .mu.M CaCl.sub.2.

US-PAT-NO: 6623948

DOCUMENT-IDENTIFIER: US 6623948 B1

TITLE: Nucleic acid sequences encoding alkaline alpha-amylases

DATE-ISSUED: September 23, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Outtrup; Helle	Vaerlose	N/A	N/A	DK
Hoeck; Lisbeth Hedegaard	Frorup	N/A	N/A	DK
Nielsen; Bjarne Ronfeldt	Virum	N/A	N/A	DK
Borchert; Torben Vedel	Copenhagen	N/A	N/A	DK
Nielsen; Vibeke Skovgaard	Bagsvaerd	N/A	N/A	DK
Bisg.ang.rd-Frantzen; Henrik	Bagsvaerd	N/A	N/A	DK
Svendsen; Allan	Birkerod	N/A	N/A	DK
Andersen; Carsten	Vaerlose	N/A	N/A	DK

APPL-NO: 09/ 540715

DATE FILED: March 31, 2000

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of U.S. application Ser. No. 09/291,023 filed on Apr. 13, 1999, now U.S. Pat. No. 6,309,871 and claims priority under 35 U.S.C. 119 of Danish application PA 1999 00438 filed on Mar. 31, 1999, the contents of which are fully incorporated herein by reference.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DK	1999 00438	March 31, 1999

US-CL-CURRENT: 435/202, 435/252.3 , 435/254.11 , 435/320.1 , 435/325 , 435/419 , 536/23.1 , 536/23.2 , 536/23.7

ABSTRACT:

The present invention relates to isolated nucleic acid sequences encoding polypeptides having alpha-amylase activity [E.C. 3.2.1.1], which may be derived from Bacillus. The invention also relates to nucleic acid constructs, vectors, and host cells comprising the nucleic acid sequences as well as methods for producing and using the polypeptides.

13 Claims, 9 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 9

---------- KWIC ---------

US Patent No. - PN (1):

6623948

Detailed Description Text - DETX (59):
Improved Ca.sup.2+ Stability of AAI-10 and AAI-6 Variant at pH 8-10.5

Detailed Description Text - DETX (78):
In relation to the above, a further aspect of the present invention relates to a method for generating a variant of a parent alpha-amylase, e.g. wherein the variant exhibits altered or increased thermal stability relative to the parent, the method comprising: (a) subjecting a DNA sequence encoding the parent alpha-amylase to random mutagenesis, (b) expressing the mutated DNA sequence obtained in step (a) in a host cell, and (c) screening for host cells expressing an alpha-amylase variant which has an altered property (e.g., thermal stability) relative to the parent alpha-amylase.

Detailed Description Text - DETX (98):
In the vector, the DNA sequence should be operably connected to a suitable promoter sequence. The promoter may be any DNA sequence, which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. Examples of suitable promoters for directing the transcription of the DNA sequence encoding an alpha-amylase variant of the invention, especially in a bacterial host, are the promoter of the lac operon of E. coli, the Streptomyces coelicolor agarase gene dagA promoters, the promoters of the Bacillus licheniformis alpha-amylase gene (amyL), the promoters of the Bacillus stearothermophilus maltogenic amylase gene (amyM), the promo-ters of the Bacillus amyloliquefaciens alpha-amylase (amyQ), the promoters of the Bacillus subtilis xylA and xylB genes etc. For transcription in a fungal host, examples of useful promo-ters are those derived from the gene encoding A. oryzae TAKA amylase, Rhizomucor miehei aspartic proteinase, A. niger neu-tral alpha-amylase, A. niger acid stable alpha-amylase, A. niger glucoamylase, Rhizomucor miehei lipase, A. oryzae alkaline protease, A. oryzae triose phosphate isomerase or A. nidulans acetamidase.

Detailed Description Text - DETX (190):
Amylases: Suitable amylases (.alpha. and/or .beta.) include those of bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Amylases include, for example, .alpha.-amylases obtained from Bacillus, e.g. a special strain of B. licheniformis, described in more detail in GB 1,296,839.

Detailed Description Text - DETX (268):
The random mutagenesis may be carried out by the following steps: 1. Select regions of interest for modification in the parent enzyme, 2. Decide on mutation sites and non-mutated sites in the selected region, 3. Decide on which kind of mutations should be carried out, e.g., with respect to the desired stability and/or performance of the variant to be constructed, 4. Select structurally reasonable mutations, 5. Adjust the residues selected by step 3 with regard to step 4. 6. Analyze by use of a suitable dope algorithm the nucleotide distribution. 7. If necessary, adjust the wanted residues to genetic code realism, e.g., taking into account constraints resulting from the genetic code, e.g., in order to avoid introduction of stop codons; the skilled person will be aware that some codon combinations cannot be used in practice and will need to be adapted 8. Make primers 9. Perform random mutagenesis by use of the primers 10. Select resulting glucoamylase variants by screening for the desired improved properties.

Detailed Description Text - DETX (272):
The assay can be used to screening of alpha-amylase variants having an

improved stability at high pH compared to the parent enzyme, and alpha-amylase variants having an improved stability at high pH and medium temperatures compared to the parent enzyme depending of the screening temperature setting

Detailed Description Text - DETX (350):
Testing the Calcium Stability of AAI-10 Variants

Detailed Description Text - DETX (351):
The calcium stability of AAI-10 variants is tested using the assays described in the "Materials and Method" section.

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  * * * * * * * * * * * * * * STN  Columbus  * * * * * * * * * * * * * * *

FILE 'HOME' ENTERED AT 09:40:33 ON 23 MAR 2004

=> fil .bec
COST IN U.S. DOLLARS                                    SINCE FILE      TOTAL
                                                           ENTRY    SESSION
FULL ESTIMATED COST                                         0.21       0.21

FILES 'MEDLINE, SCISEARCH, LIFESCI, BIOTECHDS, BIOSIS, EMBASE, HCAPLUS, NTIS,
       ESBIOBASE, BIOTECHNO, WPIDS' ENTERED AT 09:40:44 ON 23 MAR 2004
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11 FILES IN THE FILE LIST

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FILE 'MEDLINE'
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         20695 AMYLASE#
L1        4578 ALPHA AMYLASE#
                 (ALPHA(W)AMYLASE#)

FILE 'SCISEARCH'
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         16677 AMYLASE#
L2        7487 ALPHA AMYLASE#
                 (ALPHA(W)AMYLASE#)

FILE 'LIFESCI'
        151804 "ALPHA"
          4351 AMYLASE#
L3        2614 ALPHA AMYLASE#
                 ("ALPHA"(W)AMYLASE#)

FILE 'BIOTECHDS'
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          5202 AMYLASE#
L4        3237 ALPHA AMYLASE#
                 (ALPHA(W)AMYLASE#)

FILE 'BIOSIS'
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L5        9791 ALPHA AMYLASE#
                 (ALPHA(W)AMYLASE#)

FILE 'EMBASE'
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L6        3370 ALPHA AMYLASE#
                 ("ALPHA"(W)AMYLASE#)

FILE 'HCAPLUS'
       1465241 ALPHA
         43408 AMYLASE#
L7       17989 ALPHA AMYLASE#
                 (ALPHA(W)AMYLASE#)

FILE 'NTIS'
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           163 AMYLASE#
L8          60 ALPHA AMYLASE#
                 (ALPHA(W)AMYLASE#)
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FILE 'ESBIOBASE'
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L9        1909 ALPHA AMYLASE#
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FILE 'BIOTECHNO'
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L10       2130 ALPHA AMYLASE#
                 (ALPHA(W)AMYLASE#)

FILE 'WPIDS'
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L11       2197 ALPHA AMYLASE#
                 (ALPHA(W)AMYLASE#)

TOTAL FOR ALL FILES
L12      55362 ALPHA AMYLASE#

=> s l12(3a)(muta? or variant#)
FILE 'MEDLINE'
        435730 MUTA?
         94847 VARIANT#
L13         65 L1 (3A)(MUTA? OR VARIANT#)

FILE 'SCISEARCH'
        416181 MUTA?
        101104 VARIANT#
L14         81 L2 (3A)(MUTA? OR VARIANT#)

FILE 'LIFESCI'
        196238 MUTA?
         32470 VARIANT#
L15         56 L3 (3A)(MUTA? OR VARIANT#)

FILE 'BIOTECHDS'
         37338 MUTA?
         11889 VARIANT#
L16        121 L4 (3A)(MUTA? OR VARIANT#)

FILE 'BIOSIS'
        486547 MUTA?
         99467 VARIANT#
L17        155 L5 (3A)(MUTA? OR VARIANT#)

FILE 'EMBASE'
        359440 MUTA?
         82400 VARIANT#
L18         56 L6 (3A)(MUTA? OR VARIANT#)

FILE 'HCAPLUS'
        445375 MUTA?
         92446 VARIANT#
L19        280 L7 (3A)(MUTA? OR VARIANT#)

FILE 'NTIS'
          9567 MUTA?
          4451 VARIANT#
L20          0 L8 (3A)(MUTA? OR VARIANT#)

FILE 'ESBIOBASE'
        209630 MUTA?
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         35959 VARIANT#
L21         39 L9 (3A)(MUTA? OR VARIANT#)

FILE 'BIOTECHNO'
        242571 MUTA?
         41198 VARIANT#
L22         48 L10(3A)(MUTA? OR VARIANT#)

FILE 'WPIDS'
         24207 MUTA?
         23140 VARIANT#
L23         59 L11(3A)(MUTA? OR VARIANT#)

TOTAL FOR ALL FILES
L24        960 L12(3A)(MUTA? OR VARIANT#)

=> s l12(3a)(bacillus or termamyl)
FILE 'MEDLINE'
         46306 BACILLUS
             8 TERMAMYL
L25        429 L1 (3A)(BACILLUS OR TERMAMYL)

FILE 'SCISEARCH'
         45089 BACILLUS
            44 TERMAMYL
L26        645 L2 (3A)(BACILLUS OR TERMAMYL)

FILE 'LIFESCI'
         24050 BACILLUS
            13 TERMAMYL
L27        441 L3 (3A)(BACILLUS OR TERMAMYL)

FILE 'BIOTECHDS'
         15982 BACILLUS
            54 TERMAMYL
L28        923 L4 (3A)(BACILLUS OR TERMAMYL)

FILE 'BIOSIS'
         64790 BACILLUS
            66 TERMAMYL
L29        953 L5 (3A)(BACILLUS OR TERMAMYL)

FILE 'EMBASE'
         33321 BACILLUS
            24 TERMAMYL
L30        428 L6 (3A)(BACILLUS OR TERMAMYL)

FILE 'HCAPLUS'
         79183 BACILLUS
           334 TERMAMYL
L31       2018 L7 (3A)(BACILLUS OR TERMAMYL)

FILE 'NTIS'
          1637 BACILLUS
             0 TERMAMYL
L32          4 L8 (3A)(BACILLUS OR TERMAMYL)

FILE 'ESBIOBASE'
         13361 BACILLUS
            16 TERMAMYL
L33        219 L9 (3A)(BACILLUS OR TERMAMYL)

FILE 'BIOTECHNO'
         19958 BACILLUS
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            17 TERMAMYL
L34         384 L10(3A)(BACILLUS OR TERMAMYL)

FILE 'WPIDS'
         11596 BACILLUS
            35 TERMAMYL
L35         184 L11(3A)(BACILLUS OR TERMAMYL)

TOTAL FOR ALL FILES
L36        6628 L12(3A)(BACILLUS OR TERMAMYL)

=> s l24 and l36
FILE 'MEDLINE'
L37          22 L13 AND L25

FILE 'SCISEARCH'
L38          24 L14 AND L26

FILE 'LIFESCI'
L39          18 L15 AND L27

FILE 'BIOTECHDS'
L40          59 L16 AND L28

FILE 'BIOSIS'
L41          45 L17 AND L29

FILE 'EMBASE'
L42          20 L18 AND L30

FILE 'HCAPLUS'
L43         125 L19 AND L31

FILE 'NTIS'
L44           0 L20 AND L32

FILE 'ESBIOBASE'
L45           8 L21 AND L33

FILE 'BIOTECHNO'
L46          13 L22 AND L34

FILE 'WPIDS'
L47          29 L23 AND L35

TOTAL FOR ALL FILES
L48         363 L24 AND L36

=> s l48 not 1997-2004/py
FILE 'MEDLINE'
       3529531 1997-2004/PY
L49          17 L37 NOT 1997-2004/PY

FILE 'SCISEARCH'
       7042038 1997-2004/PY
L50          19 L38 NOT 1997-2004/PY

FILE 'LIFESCI'
        758896 1997-2004/PY
L51          14 L39 NOT 1997-2004/PY

FILE 'BIOTECHDS'
        123039 1997-2004/PY
L52          34 L40 NOT 1997-2004/PY
```

FILE 'BIOSIS'
3941269 1997-2004/PY
L53 28 L41 NOT 1997-2004/PY

FILE 'EMBASE'
3157051 1997-2004/PY
L54 15 L42 NOT 1997-2004/PY

FILE 'HCAPLUS'
6473577 1997-2004/PY
L55 64 L43 NOT 1997-2004/PY

FILE 'NTIS'
152480 1997-2004/PY
L56 0 L44 NOT 1997-2004/PY

FILE 'ESBIOBASE'
2026918 1997-2004/PY
L57 3 L45 NOT 1997-2004/PY

FILE 'BIOTECHNO'
829801 1997-2004/PY
L58 9 L46 NOT 1997-2004/PY

FILE 'WPIDS'
5500926 1997-2004/PY
L59 1 L47 NOT 1997-2004/PY

TOTAL FOR ALL FILES
L60 204 L48 NOT 1997-2004/PY

=> log y

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	20.28	20.49

STN INTERNATIONAL LOGOFF AT 09:44:23 ON 23 MAR 2004